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Effects of Grazers and Elevated Temperature on the Dynamics of a Wastewater Algal Treatment System

Mengyuan Li

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OF A WASTEWATER ALGAL TREATMENT SYSTEM

A thesis submitted to the College of Letters and Science in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

DEPARTMENT OF EARTH AND SPACE SCIENCE

by

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April 20th, 2017

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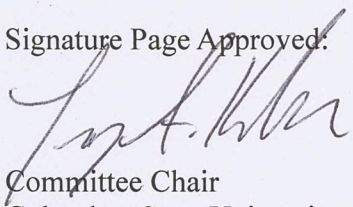

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ABSTRACT

Wastewater algal treatment systems (ATS) have been shown to effectively remove excessive nutrients from wastewater and prevent eutrophication. However, the performance of ATS could be strongly affected by environmental factors. This study examined (1) the effect of grazers on nutrient removal rates and (2) the effect of 1.7 °C increase in temperature on algal biomass. The effect of grazers was assessed outdoors by analyzing dissolved and tissue nutrient concentrations in ATS with and without biological pesticide in two 20-day trials. The effect of elevated temperature was evaluated indoors by comparing algal biomass between heated and non-heated ATS for 20 days. Grazing had no consistent, detectable effect on nutrient removal rates or periphyton tissue nutrients. Furthermore elevated temperature had no detectable effect on algal growth rates. I conclude that the performance of recirculating ATS is unlikely to be affected by grazers or slight variations in temperature if harvesting regularly (<20 days).

INDEX WORDS: Eutrophication, Climate Change, Algal Treatment System, Chironomids.

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CHAPTER ONE

INTRODUCTION

Water quality degradation is a major environmental issue in most countries of the world (Londo *et al.* 2015; Djekovic *et al.* 2016; Rivers-Moore 2016). Among other causes, the discharge of excess nutrient from poorly treated municipal sewage is one of the well-identified sources of nutrients that result in cultural eutrophication. Eutrophication generally leads to massive growth of algae and cyanobacteria in aquatic environments, which is the result of great nitrogen (N) flux from both natural (Inglett *et al.* 2011) and anthropogenic (Howarth 2008) sources. Phosphorus (P) reinforces eutrophication, especially in lakes, reservoirs, and the upper reaches of estuaries (Correll 1998).

One important source of N and P can come from urban wastewater discharges since it often contains high concentration of N and P (Ansola *et al.* 1995; Shaker *et al.* 2015; Vaillant *et al.* 2002). According to the United States Environmental Protection Agency (2004), nutrients can be removed in the tertiary treatment process in the wastewater treatment facility; however, 70% of the facilities in the US are not equipped with tertiary treatment (EPA 2004). In addition, for N and especially for P only a limited range of treatment technologies can generate effluent that meets most existing standards (von Sperling & de Lemos Chernicharo 2002). These tertiary treatment processes generally involve high costs, complex operations, and wasteful bi-products (Woods *et al.* 1999; Ko *et al.* 2004). Therefore, numerous studies have focused on the development of suitable, inexpensive and efficient wastewater treatment technologies for nutrient removal (Hoffmann 1998; Pizarro *et al.* 2006; Woertz *et al.* 2009; Li *et al.* 2011).

Six decades of research on algal-based wastewater treatment systems confirm that algae cultivation in wastewater can significantly contribute to the management and restoration of

aquatic ecosystems (Oswald & Gotaas 1957). Algal treatment systems (ATS) provide efficient means to reduce nutrient loading from both point and non-point sources (Adey *et al.* 2011). Algae not only remove nutrients from contaminated water sources (Adey *et al.* 2013), but generate biomass that can be used to create biofuels and other valuable materials (Wolkers *et al.* 2011).

One way that algae remove nutrients from wastewater is through biological uptake (Tam & Wong 2000; Liu *et al.* 2016). Through this process, algae store the nutrients intracellularly. The algae, rich in nutrients, are then harvested from the ATS to remove the nutrients. Research on other systems has demonstrated that consumers of algae (i.e., grazers) can influence algal nutrient contents and nutrient cycling in aquatic systems (Elser 1992; Hillebrand & Kahlert 2001). Knoll *et al.* (2009) showed that the interaction between herbivores and primary producers is complex in natural streams. Grazers can have both direct effects on periphyton biomass and indirect effects on nutrient dynamics in periphyton (Knoll *et al.* 2009). Grazers significantly reduced overall algal biomass through ingestion, however, periphyton nutrient content increased when algal biomass was removed. This counter-intuitive result may be caused by a reduction in competition among algal species causing an increase in the available nutrients for the remaining algae (Liess & Hillebrand 2004; Urabe 1993).

As practical and effective as ATS are, their open system design makes them vulnerable to colonization by pests such as chironomids (Craggs 2001). Chironomids, i.e. midges, are non-biting Diptera (i.e., flies), which are often the most widely distributed and abundant insects in aquatic ecosystem (Armitage *et al.* 1995). Midges are capable of thriving in stressed conditions due to their resistance to pollutants and ability to inhabit low-oxygen environments (Failla *et al.* 2014). Midge larvae can become pests when they occur at high abundances in freshwater lakes,

rivers, or municipal wastewater treatment facilities (Ali 1991). In addition, midge larvae can greatly affect plants and other organisms in their environment by consuming algae, plant matter, and invertebrates (Failla *et al.* 2014). Large midge populations could reduce the performance of ATS by decreasing the efficiency of nutrient removal and biomass production (Craggs 2001).

Various methods exist to control for midge larval populations. Chemical insecticides are among the most frequently used methods (Ali 1991; Failla *et al.* 2014), however chemical residuals may degrade water quality (Nasrabadi *et al.* 2011) and adversely affect non-target organisms (Brittain *et al.* 2010). In addition to chemical pesticides, physical and biological methods have also been employed (Failla *et al.* 2014). Keller and Husted (2015) reported that short-term dewatering in ATS significantly reduced larval midges, but algal biomass was also negatively affected by dewatering. The microbial agent, *Bacillus thuringiensis* var *israelensis* (*Bti*), has been shown to be effective as a control for other Diptera such as mosquitoes (Gwal *et al.* 2015; Dambach *et al.* 2014). *Bti* is considered to be safe for humans and animals due to the fact that its toxins are specific to Dipteran larvae. This specificity makes it an effective insecticide for reducing mosquito populations (Mehrabi *et al.* 2015). Fayolle *et al.* (2015) found that *Bti* has no impact on algae abundance or community structure. Thus, it provides a convenient and effect treatment to study how the exclusion of midges affects ATS performance.

The goal of this study is to assess the nutrient dynamics in recirculating ATS with and without midge grazing pressure. To achieve this goal, three specific objectives were established: (1) to determine the effectiveness of a recirculating ATS to remove nutrients from wastewater; (2) to ascertain whether midges affect nutrient removal performance of the ATS; (3) and to determine if midges altered periphyton tissue nutrient concentrations. I hypothesized that the ATS would significantly reduce N and P from wastewater; that midges would diminish ATS

nutrient removal performance; and that midges would enhance periphyton tissue N and P concentrations by lowering plant biomass and by increasing the relative amount of nutrients available for the remaining algae. I tested these hypotheses in two 20-day experiments that compared overall dissolved N and P removal rate and periphyton tissue nutrients between an ATS treated with pesticides (*Bti*) and one without *Bti* and ambient midge population.

METHODS

Study sites

The midge removal experiments were conducted at the Columbus Water Works, Inc. (CWW) wastewater treatment plant in Columbus (32.41°N, 84.98 °W), GA, USA. This facility treats 266,000-304,000 m^3 wastewater/day. The facility was built in 1964 as a primary treatment plant and was later upgraded to include secondary treatment.

Experiments were conducted in two groups of recirculating ATS. Each group consisted of a constant pressure head tank (208 liters), 8 vinyl flumes (i.e., floways), and a sump (568 liters) with a submersible pump to circulate water through the system (Figure 1). Each floway was 3 m long and 0.1 m wide with 0% slope. Floways were lined with unglazed, gray and blue ceramic tiles (5×5 cm) that were covered with black, fiberglass screens (1mm mesh) to create substrate for periphyton attachment. To minimize disturbance caused by heavy rains, both groups of floways were covered in a 5mm Plexiglas sheet. All floways (n=16) were adjusted to the same flow rate using a glass pitot tube. Discharge was not measured during this experiment; however, previous studies conducted on the same experimental system found floway discharge averaged 7.71±0.24 L/min (~13 cm/s). Both ATS were filled with coarse filtered (0.5 mm mesh), secondarily treated wastewater drawn directly from the CWW clarifiers (prior to pH stabilization and chlorination).

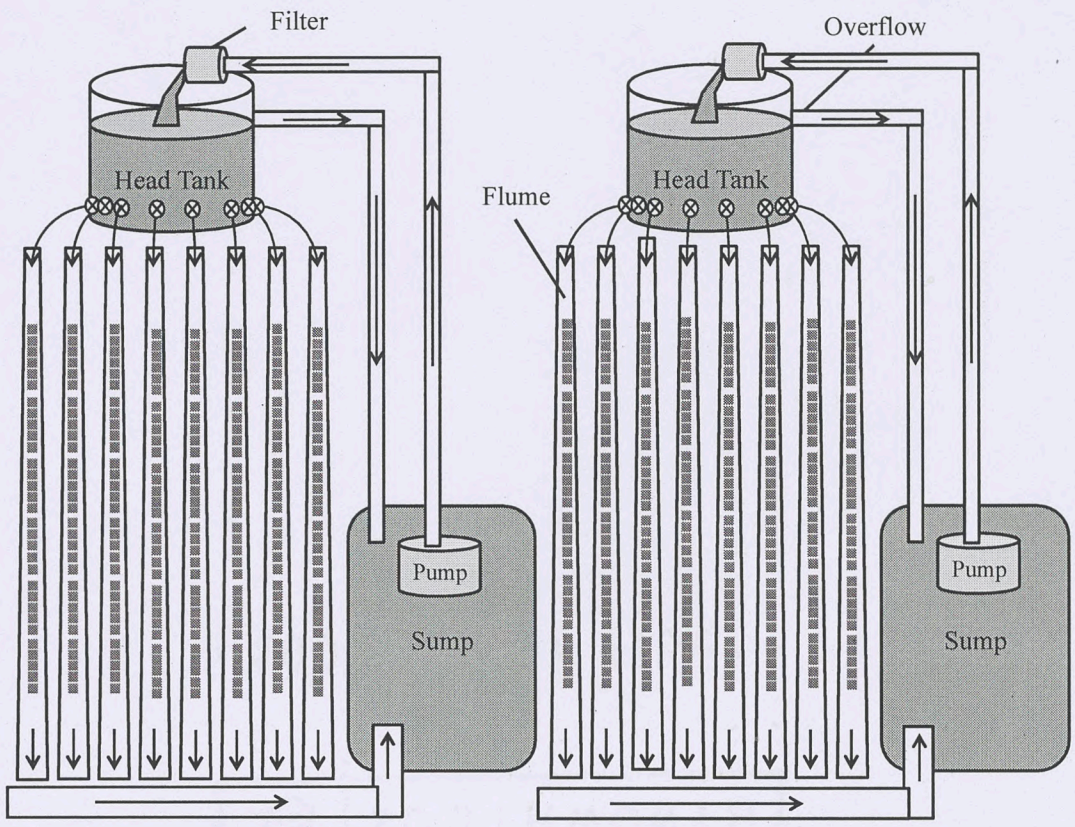

Figure 1. Diagram of recirculating ATS. Each ATS has 8 replicate floways. Wastewater runs through floways by gravity feed into the sump and is transported to the head tank by the submersible pump.

Experimental design and sampling protocols

To assess whether midges influence nutrient removal and periphyton tissue nutrient concentrations, N and P were measured in ATS water and periphyton tissue in floways operated without (i.e. control) and with *Bti* (i.e. treatment). *Bti* was applied at 10 mg/L in the treated ATS. To reach the desired concentration, seventy-eight grams of Summit Mosquito Dunks *Bti* (7000 international toxic units [ITU]/mg) were ground using mortar and pestle. The ground *Bti* was placed in 3 cloth bags (26 g/bag) and deployed in the sump of the treatment ATS.

Because each ATS recirculates the same water to all 8 floways, each ATS was assigned either the treatment (i.e., midge exclusion) or the control (i.e., midge presence). To reduce the likelihood of spurious results, the experiment was conducted twice with the treatment and control assignments reversed the second time. The first trial ran from September 13, 2015 to October 3, 2015 (20 days) and the second trial ran from October 7, 2015 to October 27, 2015 (20 days). Both trials were conducted in fall during a time when air temperature dropped and day length shortened.

Water samples were collected every four days beginning before the start of the experiment (day 0). At each sampling event, four replicates per sump were collected using 125-mL polypropylene, acid-washed sampling bottles, transported on dry ice, and stored at 4 °C until analysis. Similarly, algal samples were collected for nutrient analysis every four days starting on day 4 to provide time for the periphyton community to develop on the tiles. In each floway, tiles (n=35) were numbered and grouped into blocks of five tiles. One block of five tiles was collected at each sampling event; the sampling block was randomly selected prior to each collection and kept the same for each floway during a sampling event. Tiles within each block were randomly assigned to be analyzed for tissue nutrients, midge abundance, chlorophyll, ash free dry mass

(AFDM), and algae identification. Chlorophyll and AFDM were preserved for another research project focused on algal biomass. Tiles were placed in separate, labeled Whirl-Pak® bags, transported on dry ice, and stored frozen (-20 °C) until analysis.

A YSI 6920-V2 multi-parameter water quality sonde was deployed in each sump to record water temperature, pH, and specific conductance. The instruments logged readings hourly throughout the course of the experiment. To ensure the accuracy of the measurements, pH and conductivity sensors were calibrated using standard solutions prior to each trial. Two HOBO Pendant temperature/light loggers were deployed in each ATS to continuously record light intensity received within the systems, one of the sensors was installed in the floway in the middle of the ATS and the other was placed on the side of the system. The sensors logged readings hourly throughout the course of the experiment.

Sample analysis protocols

To assess midge abundance, previously frozen tiles were scraped with a toothbrush and rinsed with 70% ethanol before analysis. All scrapings were placed in a gridded (4 cm^2), circular petri dish (diameter 14 cm, depth 1.5 cm). Midges were counted at 12×magnification using a stereo light microscope. Only midge head capsules were counted to minimize double counting individuals damaged in the sample processing.

Water samples were stored and analyzed within 24 h after sampling following Hach® guidelines. However, due to an inadequate supply of chemicals, two sets of samples collected for the midge removal experiment (9/13, 9/25) were preserved using 18.4 M sulfuric acid. According to Hach® sample storage protocols, preserved samples can be stored up to 28 days. Within 7 days, these preserved samples were neutralized by added 5 N sodium hydroxide.

To assess ATS nutrient removal effectiveness, concentrations of nitrate (NO_3^-), nitrite (NO_2^-), ammonia (NH_3), total nitrogen (TN), total phosphorus (TP), and orthophosphate (PO_4^{3-}) were quantified following the spectrophotometric nutrient analysis protocols developed by Hach® (Method 8171, 8507, 8038, 10071, 8190, and 8048). To establish accuracy and precision of the measurement, Hach® Wastewater Effluent Inorganics Quality Control Standard was used to assess NO_3^-, NH_3, and PO_4^{3-} determinations by following the same analysis protocols (Method 8171, 8038, and 8048) but replacing sample with standard solution. Control samples were re-analyzed if measured standard concentration exceeded 10% of the known concentration. One standard was tested for every 8 samples.

To assess periphyton nutrient composition, periphyton was scraped from tiles into pre-weighed aluminum weigh dish and dried at >60°C for 48 h. Algae parts were then ground using mortar and pestle and 0.025 g was measured using an analytical balance. Dried and ground tissue nutrients were extracted following an alkaline persulfate digestion method (Smart *et al.* 1983) with slight modifications. Fifty mL digestion mixture was added to sample instead of 150 mL because Purcell and King (1996) found that 50 mL of the same digestion mixture had over 90% nutrient recovery on plant materials. Nitrate and phosphate were then analyzed colorimetrically using Hach® Method 8171 and 8048. The digestion solution was prepared daily.

To identify the dominant algal taxa growing in the ATS, previously frozen tiles were scraped with a toothbrush and rinsed with de-ionized water before analysis. All scrapings were retained in the Whirl-Pak® bags. Four drops of the sample were placed on a clean, glass slide and gently covered with a cover slip. Each prepared specimen was than examined at 400× magnification using a compound light microscope. The algae identifications were based on (Hauer & Lamberti 2007). Because the purpose of this analysis was to assess qualitatively the

dominant groups in the system, only 3 floways/treatment from the last sampling event were randomly chosen for the analysis.

To estimate periphyton biomass, periphyton was scrubbed from the tiles using a nylon bristled brush and filtered through pre-rinsed, pre-combusted, and pre-weighed GF/F filters following the Environmental Sciences Section (ESS) method 340.2 (EPA 1993). Samples were dried at 105 °C for a minimum of 12 h before weighing for dry mass.

Statistical analyses

Differences in physical measurements between treatments (*Bti* vs. control) and trials were analyzed using a repeated measures analysis of variance (RM ANOVA) for each dependent variable (water temperature, pH, specific conductance, and light intensity). To reduce autocorrelation of the data, one measurement was selected every 4 h starting from midnight (0:00, 4:00, 8:00, 12:00, 16:00, 20:00) each day yielding 240 replicates/parameter (12 replicates/day for 20 days).

To assess whether midge populations differed between treatments and trials, I used a two-way ANOVA with treatment and trial as independent variables because two-way ANOVA measures mean difference between two groups of each independent variable. Within each trial, 40 replicates/treatment (8 replicates/sample day for five sample dates) were included in the analysis.

To determine whether grazers influenced nutrient removal throughout the course of the experiment, I used separate RM ANOVA to assess differences in concentrations of TN, TP, NO_2^-, NO_3^-, NH_3, and PO_4^{3-} between treatments (*Bti* vs. control) and among dates. Tukey HSD tests were used for all post hoc pairwise comparisons because they correct the number of comparisons.

Within each trial, there were six sample dates included in the analysis and 4 replicates/sample day included in each treatment (n=24).

In order to determine the effect of grazing on periphyton tissue nutrients in the systems, I used RM ANOVA for each dependent variable (periphyton tissue N and P concentrations) with date and treatment as independent variables. To assess the unit and overall tissue nutrients in each floway, periphyton tissue N and P were expressed as concentrations/mass (mg/g) and concentrations/tile area (mg/cm^2). Concentration/tile area was calculated by multiplying concentrations/mass (mg/g) by periphyton dry mass (mg/cm^2). Tukey HSD tests were used for all post hoc pairwise comparisons. Within each trial, five sample dates were included in the analysis and 8 replicates/sample day were analyzed in each treatment (n=40).

All statistical analyses were conducted using IBM SPSS Statistics for Windows (21.0). Alpha was set to 0.05.

RESULTS

Physical measurements

To examine whether physical characteristics were consistent during the experiments, I compared water temperature, pH, specific conductance, and light intensity between treatments (*Bti* vs. control) and trials (Table 1 & 2). Specific conductance was on average higher by 63 µS/cm in trial one and 60 µS/cm in trial two in *Bti* treatments relative to controls (RM ANOVA, $F_{1,238}=1691.5$, $p<0.001$). However, temperature (RM ANOVA, $F_{1,238}=0.44$, $p=0.508$), pH (RM ANOVA, $F_{1,238}=0.83$, $p=0.363$), and light intensity (RM ANOVA, $F_{1,238}=0.001$, $p=0.971$ for floway; RM ANOVA, $F_{1,238}=0.076$, $p=0.783$ for outside) did not differ significantly between *Bti* treatments and controls.

The mean water temperature was higher in trial one than in trial two by 4.4 °C and 1.9 °C in *Bti* treatments and controls, respectively (RM ANOVA, $F_{1,238}$=130.9, p<0.001). In contrast, pH (RM ANOVA, $F_{1,238}$=22.5, p<0.001) and specific conductance (RM ANOVA, $F_{1,238}$=6053.1, p<0.001) were lower in trial one than they were in trial two. Light intensity in the floway did not differ between trials (RM ANOVA, $F_{1,238}$=2.4, p=0.123); however, light outside the floway was higher in trial two relative to trial one (RM ANOVA, $F_{1,238}$=7.3, p<0.01).

Table 1. Physical parameters in *Bti* treatments and controls in trial one. Data are means ± standard deviations.

Parameter/Treatment	*Bti*	Control
Temperature (°C)	24.43 ± 3.08	22.94 ± 3.14
pH	8.69 ± 0.64	8.37 ± 0.80
Specific conductivity (µS/cm)	528 ± 16	465 ± 10
Light intensity_floway (lum/m^2)	22,762 ± 49,866	21,744 ± 41,325
Light intensity_outside (lum/m^2)	25,449 ± 49,604	26,434 ± 51,913

Table 2. Physical parameters in *Bti* treatments and controls in trial two. Data are means ± standard deviations.

Parameter/Treatment	*Bti*	Control
Temperature (°C)	20.08 ± 4.46	21.01 ± 4.35
pH	8.71 ± 0.81	8.88 ± 0.72
Specific conductivity (µS/cm)	670 ± 11	609 ± 30
Light intensity_floway (lum/m^2)	24,819 ± 41,263	26,190 ± 50,134
Light intensity_outside (lum/m^2)	31,281 ± 51,161	33,648 ± 55,452

Algal taxonomic composition

Trial One (9/13/15-10/3/15)

Green algae and diatoms were the visually dominant groups found in both *Bti* treatments and controls. Specifically, in the *Bti* system, *Microspora*, *Cosmarium*, and *Gomphoneis* were abundant taxa, *Meridion*, *Scenedesmus*, and *Planktosphaeria* were also observed but in lower numbers. In the control system, *Cosmarium* and *Scenedesmus* were found to be most abundant, while other taxa were also identified, for example, *Microspora*, *Sorastrum*, *Protococcus*, and *Navicula*.

Trial Two (10/7/15-10/27/15)

Green algae and diatoms were still the visually dominant groups, however, I found that algae were less diverse in trial two than they were in trial one. *Scenedesmus* was the dominant green algae observed in both treatments, *Meridion*, *Microspora*, *Sorastrum*, and *Protococcus* were also found in the ATS but in much lower abundance.

***Bti* effect on midge abundance**

In order to assess how midges affect ATS treatment efficiency, I examined how well *Bti* controlled midge populations during the experiments (Figure 2; see Appendix B for figures showing *Bti* treatment effect on population density throughout 20-day experiment). Populations were reduced by 84.8% in trial one and 93.2% in trial two in *Bti* treatments relative to controls (two-way ANOVA, $F_{1,156}=148$, $p<0.001$). In addition, I also found that on average midges were less abundant in trial two than they were in trial one (two-way ANOVA, $F_{1,156}=160.6$, $p<0.001$); specifically, population density was 94.1% lower in *Bti* treatments and 86.7% lower in controls when comparing these different trials.

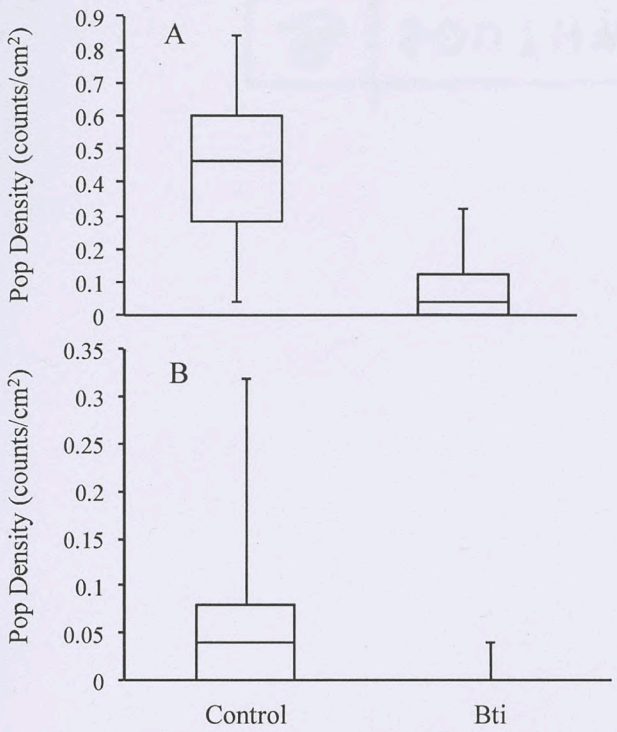

Figure 2. *Bti* treatment effect on midge population density in trial one (9/13/15-10/3/15) (A) and trial two (10/7/15-10/27/15) (B) (box lower boundary, 25th percentile; inner line, median; upper boundary, 75th percentile; whisker below, minimum; above, maximum).

Effect of *Bti* treatment on nutrient removal rates

Trial One (9/13/15-10/3/15)

All forms of N in both treatments decreased significantly over the course of this 20-day experiment (Table 3, $p<0.001$ for all). In particular, TN, nitrite, nitrate, and ammonia declined by 91%, 62%, 82%, and 75%, respectively, in the treatment sump (midge exclusion) and 97%, 90%, 73%, and 88%, respectively, in the control sump. Total nitrogen, nitrite, and nitrate gradually reduced throughout the course of the experiment (Figure 3A-3C). However, ammonia concentration dropped sharply between day 0 and day 4 of the experiment (Tukey HSD, $p<0.001$) then remained constant (Figure 3D). Although N concentrations declined significantly over time, treatment had no statistically significant effects on TN and nitrite concentrations (Table 3, $p>0.1$

for both). However, nitrate concentration was 11% lower (Table 3, p=0.002) in the treatment sump, while the pattern reversed for ammonia concentration (Table 3, p=0.006). The interaction between date and treatment was significant for nitrite (Table 3, p=0.032) and ammonia (Table 3, p=0.011), while no significant interaction effect was detected for TN (Table 3, p=0.643) and nitrate (Table 3, p=0.358). Nitrite concentration changed over time in both treatments, however, the pattern varied between *Bti* treatments and controls. Specifically, concentration was consistent between day 8 and day 16 in *Bti* treatments (Tukey HSD, p>0.1), but the same pattern was not shown in controls (Tukey HSD, p<0.005). Similarly, ammonia concentration remained consistent between day 4 and day 12 in the treatment sump (Tukey HSD, p=0.757), while it declined in the control sump (Tukey HSD, p<0.005).

By the end of the experiment, TP concentration declined by 73% and 70% in treatments and controls, respectively; phosphate was reduced by 55% and 40% in treatments and controls, respectively (Figure 3E & 3F, Table 3, p<0.001 for both). In addition, both TP and phosphate concentrations were lower in *Bti* treatment (Table 3, p<0.001 for both). There existed a significant date by treatment interaction for TP and phosphate concentrations (Table 3, p<0.001 for both). Both nutrient concentrations changed over time; however, the patterns were not consistent between treatments. For example, during the first 8 days of the experiment, TP declined linearly in the treatment sump (Tukey HSD, p<0.005), as it remained constant in the control sump (Tukey HSD, p>0.1). Phosphate concentration increased between day 4 and day 8 (Tukey HSD, p=0.023) then remained constant until day 16 (Tukey HSD, p>0.1) in controls. Conversely phosphate fluctuated greatly in *Bti* treatments (Tukey HSD, p<0.005). Overall, the mean removal of N and P was 0.36 g N m^{-2} day^{-1} and 0.26 g P m^{-2} day^{-1}, respectively, in both ATS units.

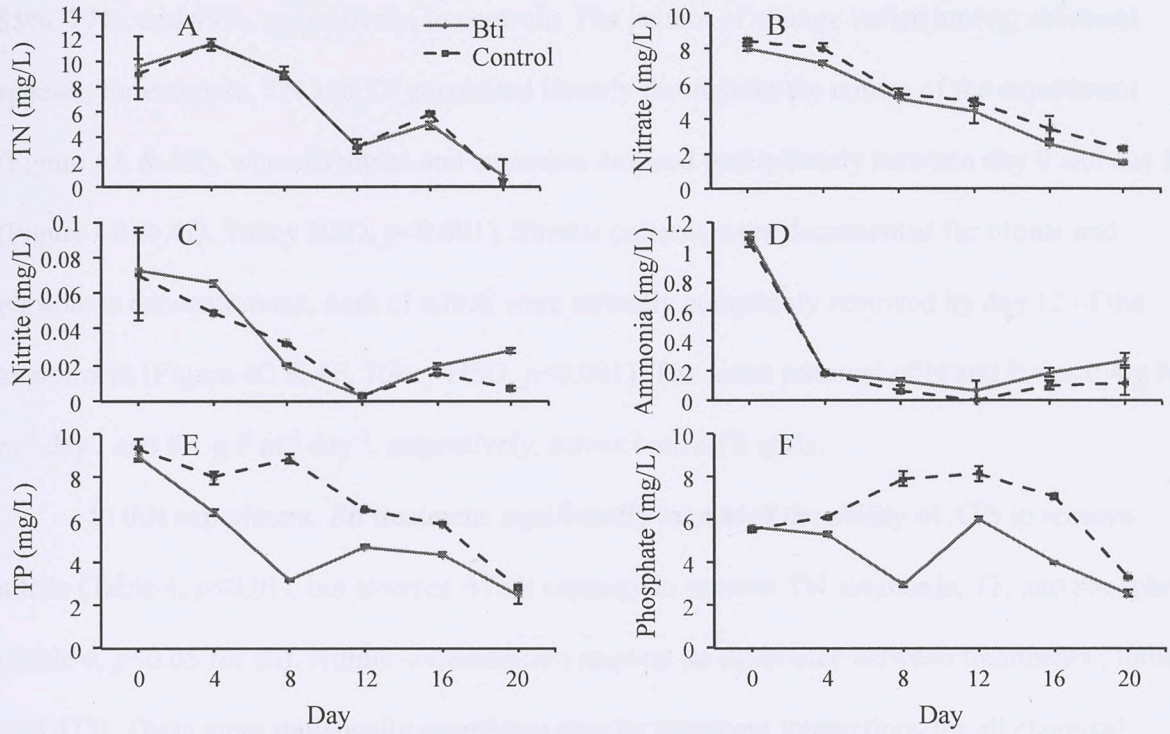

Figure 3. TN (A), nitrate (B), nitrite (C), ammonia (D), TP (E), and phosphate (F) concentrations in wastewater ATS treated with *Bti* treatments (solid line, midge exclusion) and controls without *Bti* (dashed line, midge present) in trial one (9/13/15-10/3/15). Error bars represent ± 1 standard deviation.

Table 3. Summary of repeated measures ANOVA statistical results for date, treatment, and their interaction for 6 nutrient species in a wastewater ATS during trial one (9/13/15-10/3/15).

Chemical Species	Date		Treatment		Interaction	
	$F_{5,30}$	*p*	$F_{1,6}$	*p*	$F_{5,30}$	*p*
Total Nitrogen	159.7	**<0.001**	0.001	0.977	0.679	0.643
Nitrite	56.2	**<0.001**	3.44	0.113	2.9	**0.032**
Nitrate	523.5	**<0.001**	29.2	**0.002**	1.1	0.358
Ammonia	905.2	**<0.001**	17.01	**0.006**	3.6	**0.011**
Total Phosphorus	776.8	**<0.001**	603.7	**<0.001**	160.0	**<0.001**
Phosphate	575.0	**<0.001**	2779.2	**<0.001**	277.0	**<0.001**

Trial Two (10/7/15-10/27/15)

Similar to trial one, nutrient concentrations in the wastewater ATS dropped over time (Table 4, $p<0.001$ for all). Total nitrogen, nitrate, nitrite, ammonia, TP, and phosphate declined by 83%, 99%, 99%, 83%, 66%, and 99%, respectively, in *Bti* treatments and 90%, 97%, 100%,

85%, 77%, and 99%, respectively, in controls. The pattern of change varied among chemical species, for example, TN and TP attenuated linearly throughout the course of the experiment (Figure 4A & 4E), whereas nitrite and ammonia dropped precipitously between day 0 and day 4 (Figure 4B & 4D, Tukey HSD, $p<0.001$). Similar patterns were documented for nitrate and phosphate concentrations, both of which were virtually completely removed by day 12 of the experiment (Figure 4C & 4F, Tukey HSD, $p<0.001$). The mean removal of N and P was 0.4 g N m^{-2} day^{-1} and 0.1 g P m^{-2} day^{-1}, respectively, across both ATS units.

In this experiment, *Bti* treatment significantly increased the ability of ATS to remove nitrite (Table 4, $p<0.01$), but lowered ATS's capacity to remove TN, ammonia, TP, and phosphate (Table 4, $p<0.05$ for all). Nitrate concentration showed no difference between treatments (Table 4, $p=0.473$). There were statistically significant date by treatment interactions for all chemical species measured (Table 4, $p<0.005$ for all), which indicated that the effect of *Bti* treatment on nutrient concentrations was date dependent. Specifically, TN, TP, and nitrite concentrations declined between day 4 and day 12 in the control sump (Tukey HSD, $p<0.01$ for all), while the concentrations were consistent in the treatment sump (Tukey HSD, $p>0.1$ for all). Ammonia concentration increased continuously starting from day 4 in controls (Tukey HSD, $p=0.031$) but it did not differ at any date after day 4 in *Bti* treatments (Tukey HSD, $p>0.1$). The pattern was reversed for phosphate concentration, of which declined between day 0 and day 4 in *Bti* treatments (Tukey HSD, $p<0.005$), while it stayed consistent in controls (Tukey HSD, $p>0.1$).

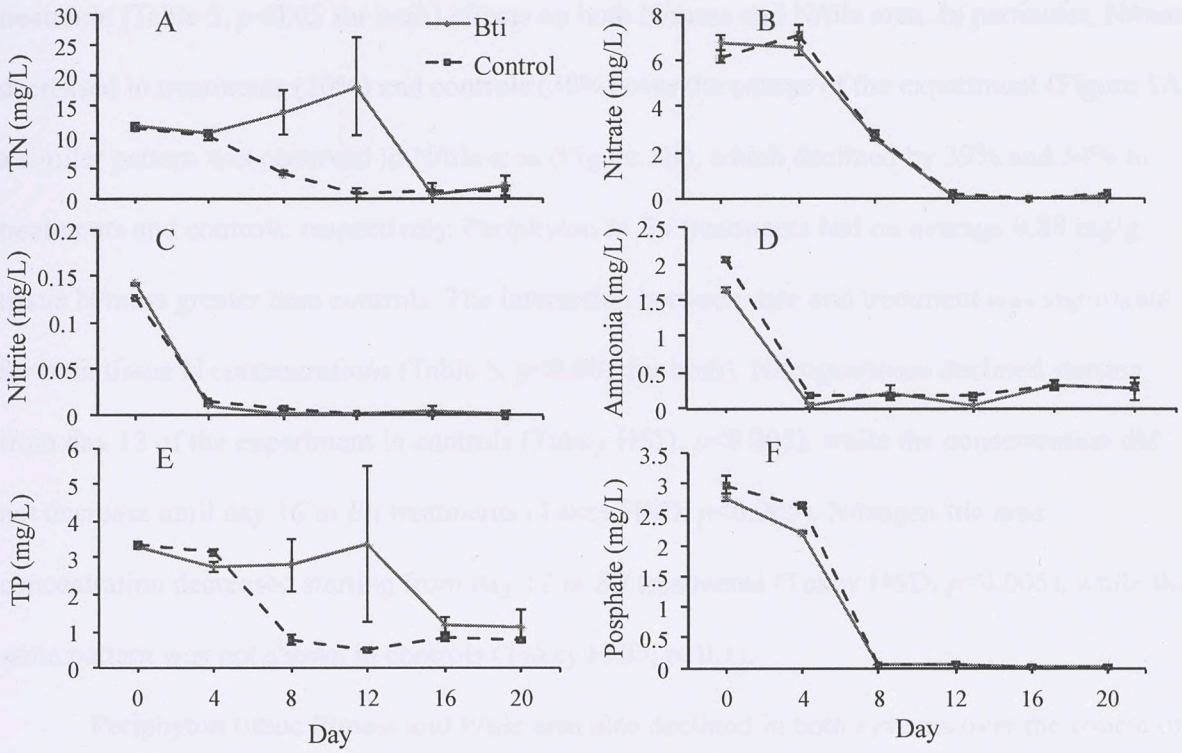

Figure 4. TN (A), nitrate (B), nitrite (C), ammonia (D), TP (E), and phosphate (F) concentrations in wastewater ATS treated with *Bti* treatments (solid line, midge exclusion) and controls without *Bti* (dashed line, midge present) in trial two (10/7/15-10/27/15). Error bars represent ± 1 standard deviation.

Table 4. Summary of repeated measures ANOVA statistical results for date, treatment, and their interaction for 6 nutrient species in a wastewater ATS during trial two (10/7/15-10/27/15).

Chemical Species	Date		Treatment		Interaction	
	$F_{5,30}$	*p*	$F_{1,6}$	*p*	$F_{5,30}$	*p*
Total Nitrogen	21.04	**<0.001**	398.5	**<0.001**	12.9	**<0.001**
Nitrite	4494.5	**<0.001**	15.2	**0.008**	22.4	**<0.001**
Nitrate	1596.1	**<0.001**	0.59	0.473	5.1	**0.002**
Ammonia	1247.1	**<0.001**	9.84	**0.020**	18.2	**<0.001**
Total Phosphorus	11.3	**<0.001**	81.2	**<0.001**	5.2	**0.002**
Phosphate	6846.8	**<0.001**	24.3	**0.003**	32.6	**<0.001**

Effect of *Bti* treatment on periphyton tissue nutrients

Trial One (9/13/15-10/3/15)

I assessed midge impact on periphyton nutrient uptake by extracting tissue N and P from the periphyton. Analyses revealed statistically significant date (Table 5, $p<0.001$ for both) and

treatment (Table 5, $p<0.05$ for both) effects on both N/mass and N/tile area. In particular, N/mass decreased in treatments (20%) and controls (38%) over the course of the experiment (Figure 5A); a similar pattern was observed in N/tile area (Figure 5B), which declined by 39% and 34% in treatments and controls, respectively. Periphyton in *Bti* treatments had on average 0.88 mg/g tissue N/mass greater than controls. The interaction between date and treatment was significant for both tissue N concentrations (Table 5, $p<0.001$ for both). Nitrogen/mass declined starting from day 12 of the experiment in controls (Tukey HSD, $p<0.005$), while the concentration did not decrease until day 16 in *Bti* treatments (Tukey HSD, $p<0.005$). Nitrogen/tile area concentration decreased starting from day 12 in *Bti* treatments (Tukey HSD, $p<0.005$), while the same pattern was not shown in controls (Tukey HSD, $p>0.1$).

Periphyton tissue P/mass and P/tile area also declined in both systems over the course of the experiment (Figure 5C & 5D, Table 5, $p<0.001$ for both). Phosphorus/mass reduced by 61% and 35% in treatments and controls, respectively; P/tile area varied in comparable pattern, of which declined by 71% and 26% in treatments and controls, respectively. *Bti* treatment had no statistically significant effect on periphyton tissue P/mass and P/tile area (Table 5, $p>0.05$ for both). However, I found a significant interaction between date and treatment for both tissue P concentrations (Table 5, $p<0.001$ for both). These results indicated that the effect of *Bti* treatment on periphyton tissue P/mass and P/tile area varied among dates. Specifically, tissue P/mass decreased starting from day 8 in controls (Tukey HSD, $p<0.005$); however, it did not start decreasing until day 12 in *Bti* treatments (Tukey HSD, $p<0.01$). Tissue P/tile area declined between day 8 and day 12 (Tukey HSD, $p<0.001$) then remained consistent (Tukey HSD, $p>0.1$) in controls, whereas in *Bti* treatments, it decreased starting from day 8 until day 16 (Tukey HSD, $p<0.001$).

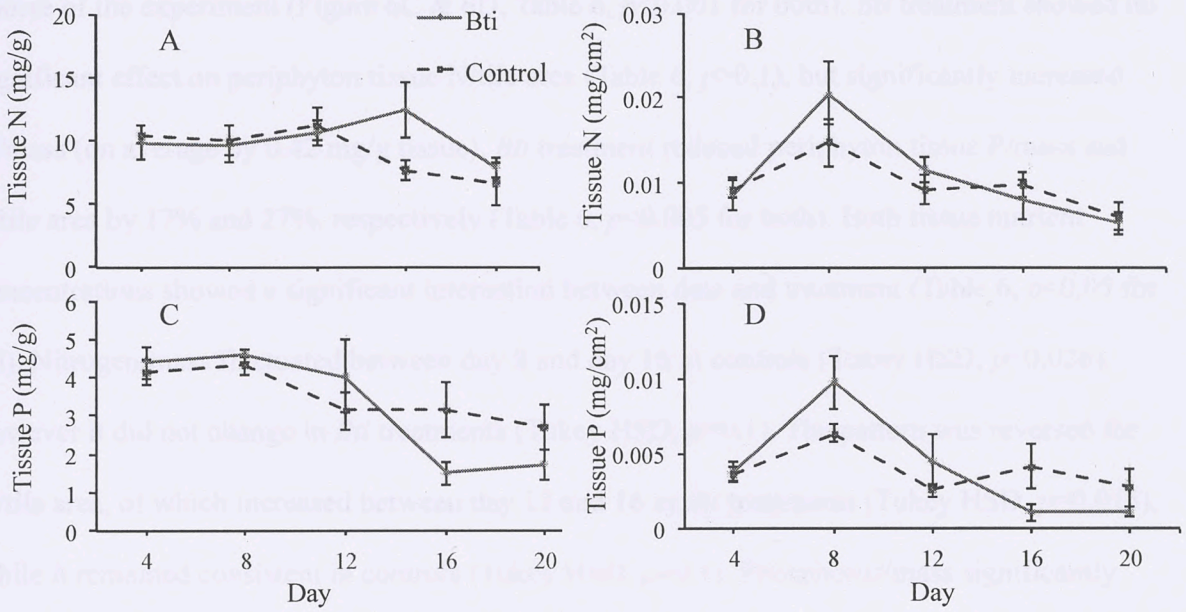

Figure 5. Periphyton tissue N/mass (A) and N/tile area (B) and periphyton tissue P/mass (C) and P/tile area (D) in wastewater ATS treated with *Bti* treatments (solid line, midge exclusion) and controls without *Bti* (dashed lines, midge present) in trial one (9/13/15-10/3/15). Error bars represent ± 1 standard deviation.

Table 5. Summary of repeated measures ANOVA statistical results for date, treatment, and their interaction for mass basis and tile area of periphyton tissue nutrients in a wastewater ATS during trial one (9/13/15-10/3/15).

		Date		Treatment		Interaction	
		$F_{4,56}$	p	$F_{1,14}$	p	$F_{4,56}$	p
Mass Basis (mg/g)	Tissue N	8.7	**<0.001**	7.5	**0.016**	10.2	**<0.001**
	Tissue P	74.4	**<0.001**	3.5	0.084	18.3	**<0.001**
Tile Area (mg/cm^2)	Tissue N	68.4	**<0.001**	5.9	**0.029**	8.1	**<0.001**
	Tissue P	94.3	**<0.001**	0.4	0.54	27.9	**<0.001**

Trial Two (10/7/15-10/27/15)

Periphyton tissue N/mass and N/tile area both varied significantly over time (Table 6, $p<0.005$ for both). Nitrogen/tile area (Figure 6B) fluctuated in both treatments over the course of the experiment; however, N/mass (Figure 6A) did not differ statistically in *Bti* treatments at any sample date (Tukey HSD, $p>0.1$). In contrast, both tissue P/mass and P/tile area significantly declined by approximately half of the original concentrations in treatments and controls over the

course of the experiment (Figure 6C & 6D, Table 6, $p<0.001$ for both). *Bti* treatment showed no significant effect on periphyton tissue N/tile area (Table 6, $p>0.1$), but significantly increased N/mass (on average by 0.42 mg/g tissue). *Bti* treatment reduced periphyton tissue P/mass and P/tile area by 17% and 27%, respectively (Table 6, $p<0.005$ for both). Both tissue nutrient concentrations showed a significant interaction between date and treatment (Table 6, $p<0.05$ for all). Nitrogen/mass fluctuated between day 8 and day 16 in controls (Tukey HSD, $p=0.026$), however it did not change in *Bti* treatments (Tukey HSD, $p>0.1$). The pattern was reversed for N/tile area, of which increased between day 12 and 16 in *Bti* treatments (Tukey HSD, $p=0.015$), while it remained consistent in controls (Tukey HSD, $p>0.1$). Phosphorus/mass significantly declined until day 8 in *Bti* treatments (Tukey HSD, $p<0.001$), while it did not differ statistically (Tukey HSD, $p>0.1$) until day 12 which concentration decreased (Tukey HSD, $p<0.01$) in controls. Tissue P/tile area also changed differently between treatments, which increased between day 4 and day 8 (Tukey HSD, $p<0.005$) then decreased between day 12 and day 16 (Tukey HSD, $p<0.01$) in controls, however, the same pattern was not observed in *Bti* treatments (Tukey HSD, $p>0.1$).

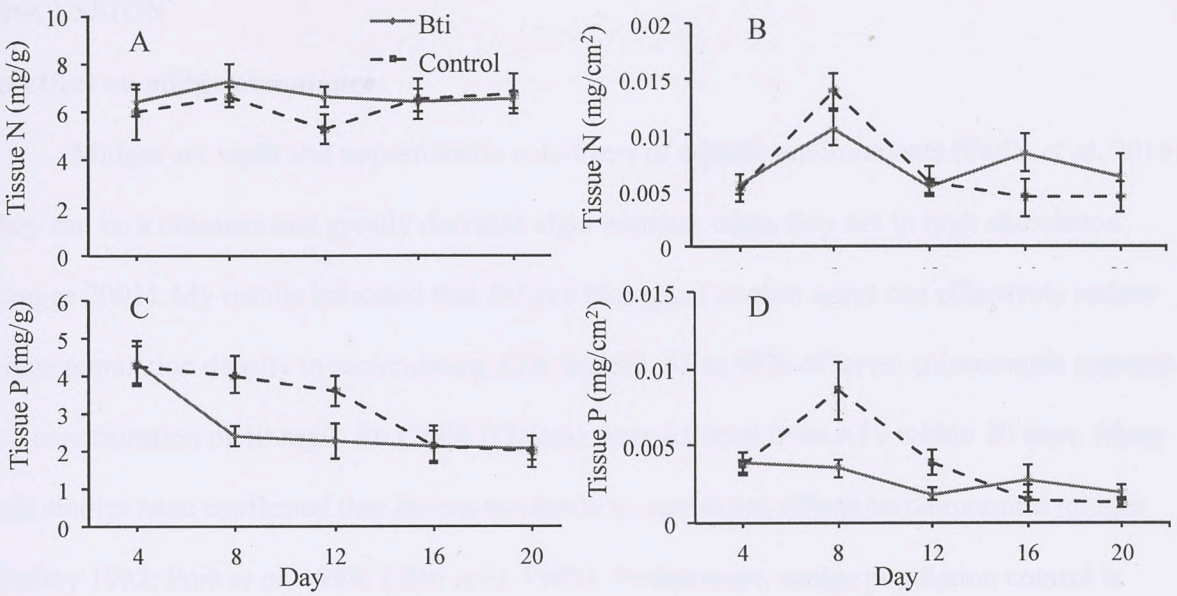

Figure 6. Periphyton tissue N/mass (A) and N/tile area (B) and algal tissue P/mass (C) and P/tile area (D) in wastewater ATS treated with *Bti* treatments (solid line, midge exclusion) and controls without *Bti* (dashed line, midge present) in trial two (10/7/15-10/27/15). Error bars represent ± 1 standard deviation.

Table 6. Summary of repeated measures ANOVA statistical results for date, treatment, and their interaction for mass basis and tile area of periphyton tissue nutrients in a wastewater ATS during trial two (10/7/15-10/27/15).

		Date		Treatment		Interaction	
		$F_{4,56}$	*p*	$F_{1,14}$	*p*	$F_{4,56}$	*p*
Mass Basis (mg/g)	Tissue N	5.3	**0.001**	4.6	**0.05**	3.6	**0.011**
	Tissue P	83.2	**<0.001**	20.9	**<0.001**	17.8	**<0.001**
Tile Area (mg/cm^2)	Tissue N	87.9	**<0.001**	1.2	0.29	18.0	**<0.001**
	Tissue P	71.4	**<0.001**	18.0	**0.001**	39.8	**<0.001**

DISCUSSION

Bti effect on midge abundance

Midges are rapid and opportunistic colonizers of aquatic environments (Failla *et al.* 2015). They can be a nuisance and greatly decrease algal biomass when they are in high abundance (Craggs 2001). My results indicated that *Bti* as a biological control agent can effectively reduce midge population density in recirculating ATS. Indeed, 85 to 93% of larval chironomids exposed to a concentration of 10 mg/L *Bti* (7000 ITU/mg) were reduced from ATS within 20 days. Many field studies have confirmed that *Bti* has moderate to significant effects on chironomid midges (Molloy 1992; Pont *et al.* 1999; Liber *et al.* 1998). Furthermore, midge population control is strongly affected by the dosage and potency of *Bti* (Rodcharoen *et al.* 1991). For example, Rodcharoen *et al.* (1991) reported 57% reduction in larval chironomids using a 600 ITU/mg *Bti* product at 7.47 mg/L throughout a period of 14 days. Stevens *et al.* (2013) documented 71 to 93% reduction using 3000 ITU/mg *Bti* at 1.46 mg/L to 4.38 mg/L over a 19-day period.

Midge distributions are highly influenced by various environmental factors, such as temperature (Eggermont & Heiri 2011), water depth (Chen *et al.* 2014), pH (Nyman *et al.* 2005), and precipitation (Matthews-Bird *et al.* 2016). In my study, even though midge populations were greatly reduced by *Bti* in both experiments, the two systems had considerably lower population density (94.1% in *Bti* treatment and 86.7% in control) in trial two than trial one. Water depth and precipitation pattern were not studied in this paper, but compared to trial one, the mean water temperature in trial two had declined by 4.4 °C and 1.9 °C in treatments and controls, respectively. The distribution and range of midges are strongly influenced by water temperature (Walker *et al.* 1991). Higher temperature decreases the time to hatch and promotes larval growth, however, growth rate declines at temperatures exceeding 28 °C (Maier *et al.* 1990). In my study,

the mean water temperature for both ATS in both trials was maintained between 20-25 °C, which did not exceed the ideal temperature range for larval growth. Therefore, there is a possibility that cold water in the second trial inhibited their hatching and growth.

Effect of *Bti* treatment on nutrient removal rates

Results from both experiments indicated that these recirculating ATS reduced N nearly 90% and P approximately 70% from wastewater. This finding was consistent with previous studies (Liu *et al.* 2016; Kebede-Westhead *et al.* 2003; Selvaratnam *et al.* 2015) which have demonstrated that an ATS can lower N by 59%-99% and P by 70%-99%. ATS are a promising technology for nutrient removal from water, as a result, it has been applied for treating polluted rivers, municipal wastewater, and dairy manure slurries (Adey *et al.* 2011; Craggs *et al.* 1996; Mulbry *et al.* 2008). Thus, my findings support the first hypothesis that ATS would significantly remove or reduce N and P.

My second hypothesis, that nutrient removal would be diminished in the control (midge presence) system due to destruction of the algal periphyton by grazers, was not supported. Grazing reduced nutrient removal rates in the first trial; however, the pattern was reversed in the second trial. The results suggest that grazing had no consistent, detectable effect on nutrient removal rates in these recirculating ATS. Montemezzani *et al.* (2016) reported that grazing pressure, which reduces algal biomass, is associated with lower nutrient removal rate. Statistical analyses of nutrient removal in trial two revealed increased nutrient removal rates in controls (associated with elevated periphyton biomass in the system). Substantial biomass in controls during the middle of the second trial (approx. day 8 - day 12) was coincident with greater nutrient removal occurred on those same days (see Appendix E for figures showing periphyton biomass in treated and control ATS units). Previous publications have confirmed that algal

biomass often decreases due to grazing pressure (Feminella & Hawkins 1995; Liess & Hillebrand 2004); alternatively, in some circumstances, grazing can have no or positive effects on algal biomass. For example, Dudley (1992) found that herbivores can be beneficial to algal growth by efficiently removing their competitors. Colletti *et al.* (1987) reported no significant difference in diatom abundance between grazed and ungrazed systems at herbivore (mayfly nymph) densities of 800 nymphs/m^2, but a decline in diatom abundance at densities of $\geq$ 2800 nymphs/m^2. In my study, midge densities were at 4460 midges/m^2 in trial one and 590 midges/m^2 in trial two. The differential midge densities might explain the inconsistent effect of grazing on nutrient removal rates.

Although nutrients were effectively removed, my experiment was conducted at a pilot-scale over a short period of time. Typically, full-scale ATS systems that run year-round, and could have mean N and P removal rates much higher than those I found in my small-scale ATS. Craggs *et al.* (1996) reported an annual average of 1.11 $\pm$ 0.48 g/m^2/day N removal and 0.73 $\pm$ 0.28 g/m^2/day P removal in a full-scale ATS installed at a wastewater unit in Patterson, CA. These removal rates were approximately three times higher than I recorded in my experiment. Algal biomass and taxonomic composition are highly influenced by environmental factors, such as temperature and light intensity. For instance, cyanobacteria prefer high temperature and high light intensity whereas many diatoms favor low temperature and require less light (Lan *et al.* 2015). To improve the effectiveness of ATS performance for removing nutrients and better understanding the systems' dynamics, it is necessary to conduct a complete analysis on full-scale ATS systems with an extended observation period combined with a more thorough analysis of the algal species growing in the ATS.

Effect of *Bti* treatment on periphyton tissue nutrients

Grazing pressure reduced periphyton tissue N concentration in the first trial, however, that same pattern was not confirmed in the second trial. Conversely, in the second trial, grazing increased periphyton tissue P accumulation, but had no effect in the first trial. These results suggest that grazing was unlikely to explain periphyton tissue nutrient accumulation in this study. These results did not support my third hypothesis that grazing would enhance cellular concentrations of both N and P by lowering plant biomass and by increasing the relative amount of nutrients available for the remaining algae.

Hillebrand *et al.* (2008) reported that grazers significantly increased periphyton N- and P-content. Frost & Elser (2002) confirmed that periphytic P-content in ungrazed treatments was significantly lower than it was at high grazing pressure; the same response was observed for N-content (Hunter & Russell-Hunter 1983). However, it has been suggested that nutrient content declines in the presence of grazers, which is consistent with the effect of grazing on algal biomass (Jacoby 1985; Mazumder *et al.* 1989). I found lower periphyton tissue N in the grazed ATS was consistent with the finding reported by Gelwick & Matthews (1992), the possible explanation to this observation is that grazers removed algal species with high N-content, which were later replaced by low N-content species (Steinman 1996).

Data from previous studies have documented that grazing substantially increased nutrient supply by recycling nutrients via excretion (Sterner 1986; Elser 1992). Thus, grazers may enhance periphyton tissue nutrient concentrations by increasing the availability of nutrients to remaining algae. However, periphyton tissue P declined over time in the ATS with midges present in my study. Hillebrand *et al.* (2008) found that the magnitude of grazer effects on periphyton nutrient content is highly influenced by the nutrient content of grazers, which implies

that nutrients could be retained in herbivore's biomass if the herbivore itself is low in nutrients. Therefore, periphyton tissue P decreased over time might be due to midge retention of P.

In contrast to the noticeable declines observed for tissue P-content, tissue N concentrations remained constant. This result is similar to that reported by Mayakun *et al.* (2013), who observed no evident change in tissue N concentration under a nutrient-enriched environment. There are few possible explanations to that. Firstly, some of the N might be depleted from the water column by denitrification, leading to the production of a variety of nitrogen gases that were volatilized to the atmosphere. Secondly, additional N might accumulate in sediments (Havens *et al.* 1999) that could be sequestered in our sumps, and thus not measured in this study.

CONCLUSION

ATS efficiently removed N and P from municipal wastewater. Furthermore, grazing pressure had no detectable effects on nutrient removal rates and periphyton tissue nutrients. My study confirms that field application of ATS has great potential for reducing nutrients from wastewater. A better understanding of the interaction between periphyton community and herbivores is needed to ensure optimal ATS nutrient removal efficiencies.

CHAPTER TWO

INTRODUCTION

Global average air temperature has risen by approximately 0.6 °C over the 20th century (IPCC 2001) and the climate models predict that it may increase 1.7° to 4.9 °C by the end of this century (Wigley & Raper 2001). These changes in climate have been forecasted to influence biotic interactions and ecosystem services (Montoya & Raffaelli 2010). Increasing temperature may affect the physiology and performance of organisms, alter the distribution and abundance of species, and lead to changes in species composition (Harley *et al.* 2006). For example, species with low tolerance to high temperature will be more vulnerable to global warming (Calosi *et al.* 2008). In addition, increasing temperature is estimated to affect drinking water and wastewater infrastructure by facilitating the growth of toxic bacteria (Paerl & Paul 2012) and increasing chances of severe weather (e.g. frequent heavy rainfall) which could in turn lead to more untreated sewer overflows (Tolkou & Zouboulis 2015). As a result, it is thus necessary to investigate species and community-level responses to increased temperature to assess how these will affect the management and restoration of ecosystems.

Climate change has many ecological and economic implications (Adams *et al.* 1990; Bakkenes *et al.* 2002; Bellard *et al.* 2012). One of the greatest challenges is that climate change is strongly affecting the Earth's water resources (Arnell 1999) by modifying stream flow and nutrient loading (Chang *et al.* 2001), altering natural hydrologic cycle, and influencing water quality and supply (Lettenmaier *et al.* 2009). The organisms living in a waterbody are subsequently affected by these changes. Algae are primitive unicellular or multicellular photosynthetic organism (Meyer & Krueger 2012) that can thrive in freshwater, seawater, and even wastewater (Adey *et al.* 2013; de Faveri *et al.* 2015; Liu *et al.* 2016). Algae have been

identified as a promising way of generating renewable biofuel due in part to their rapid growth rate, non-competitive nature to food crops, and ability to yield oil on marginal land (Lam & Lee 2012; Roberts *et al.* 2013; Chisti 2007).

Algae are not only a potential bioenergy source, but also a great medium for recycling and recovering nutrients from wastewater streams. Boelee *et al.* (2011) stated that algae could be harnessed to remove nitrogen (N) and phosphorus (P) from wastewater using their capacity to uptake nutrients into their cells. Nutrients, specifically, N and P are the fundamental components needed for algal growth and metabolism (Chavan *et al.* 2014). Nitrogen is a constituent used to construct proteins and enzymes. Phosphorus is a vital element, because it is a component of DNA and ATP (Richmond 2008). When high concentrations of N and P are discharged into natural ecosystems, it can stimulate nuisance algae blooms that ultimately result in the depletion of dissolved oxygen, and kill fish and invertebrates (Gucker *et al.* 2006). Thus, algae can be used in a tertiary treatment system (i.e., algal treatment system) to remove nutrients from wastewater to prevent cultural eutrophication, the nutrient over-enrichment of ecosystems, and all of its associated problems (Wang & Lan 2011; Higgins & Kendall 2012).

Because nutrients are generally not limiting in municipal wastewater, factors such as light intensity, temperature, pH, and dissolved inorganic carbon can limit algae growth in tertiary wastewater treatment systems (Mata *et al.* 2013). Seasonal changes in temperature greatly affect algal biomass accumulation, which in turn, influences the effectiveness of algal treatment system (ATS). Temperature is a critical factor that could slow algal growth rate, alter species composition, and shift nutrient requirements (Chavan *et al.* 2014). Algae survive across a variety of temperatures; however, many species often show thermal optima (Chen & Bern 1980). In general, warm temperate environments are more favorable to algal growth (Shilton 2005).

Keesing *et al.* (2016) found that green algae tend to have higher biomass accumulation in warmer temperature. Temperature may shift the efficiency of tertiary wastewater treatment systems, such as algal treatment systems (ATS). For example, Mulbry *et al.* (2010) reported that an ATS had higher nutrient removal rates in summer and fall than in winter due to variations in algal biomass.

In this study, I analyze temperature-algal biomass relations using a periphyton-based wastewater ATS to determine if elevated temperature (+1.7 °C above ambient) affects algal biomass accrual. I hypothesized that elevated water temperature would enhance algal growth. I tested this hypothesis in a short-term, controlled laboratory experiment that compared algal biomass accrual between ATS at elevated temperature (1.7 °C above ambient temperature) and those at ambient temperature.

METHODS

Study sites

This experiment measured how elevated temperature affects algal growth. The study was conducted in a laboratory at Columbus State University in Columbus, GA, USA (32.50 °N, 84.94 °W) to ensure that controlled conditions minimized variations in water temperature, precipitation and evaporation. Sixteen independent, recirculating floways (1.2 m long, 0.6 m high) were constructed of polyvinyl chloride pipes (5.1 cm diameter). Water was circulated through floways using compressed air discharged by air stones (2.5×1.5 cm) submerged in the vertical portion of the pipe (Figure 7). Floways were lined with unglazed, gray ceramic tiles (2.3×2.3 cm), to serve as substrate for periphyton and to facilitate algae sampling. Ten 40W full spectrum fluorescent grow lights (GE T12 49893) were placed approximately 5 cm above the

floways to provide light for algae. To mimic natural light-dark cycles, lights were cycled on and off every 12 hours. Temperature was raised by 1.7 °C above ambient in half of the floways using 25W aquarium heaters (Eheim Jager TruTemp). To reduce the possibility of positional bias, pairs of floways were randomly assigned either treatment (heated) or control (ambient). Each floway was filled with 8 L of coarse filtered (Advanced Drainage Systems, Drain-Sleeve®, 0.5 mm), secondarily treated wastewater, collected from a clarifier at the Columbus Water Works, Inc. (CWW).

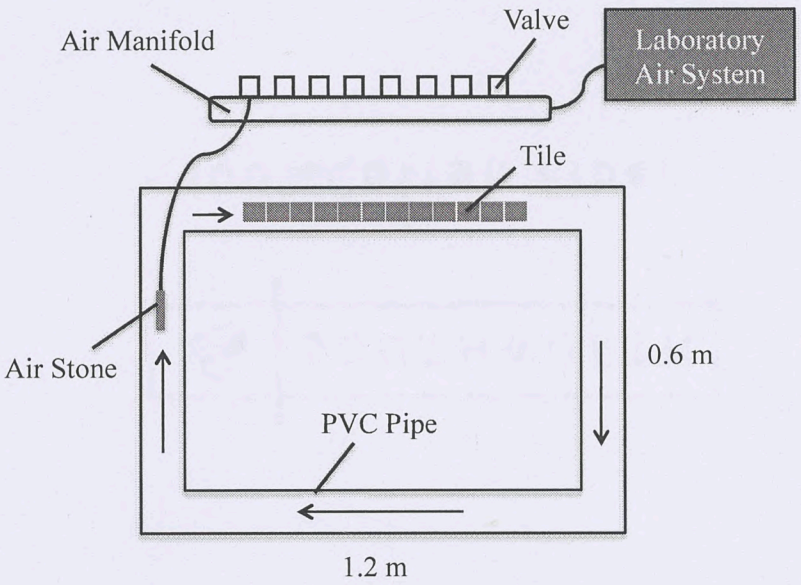

Figure 7. Diagram of a recirculating floway. Each floway is constructed of four PVC pipes. Wastewater circulates through each floway by compressed air through air stone.

Sampling protocols

The experiment was conducted from January 15, 2016 to February 4, 2016 (20 days). I assessed the influence of temperature on periphyton biomass by comparing chlorophyll a (chl-a), dry mass (DM), and ash-free dry mass (AFDM) between treatments with heaters (n=8) and controls without (n=8). Tiles were collected every four days starting on day 4. In each floway, tiles (n=16) were numbered and grouped into blocks of two tiles. One block of two tiles was chosen randomly prior to each sampling event. That same block of tiles was then sampled across all floways. The two tiles within each block were randomly assigned to be analyzed for chlorophyll pigment and DM/AFDM. Tiles were placed in separate, labeled Whirl-Pak® bags and stored frozen (-20 °C) until analysis.

Water temperature of both treatments and controls were measured in degree Celsius using Flinn digital thermometer (0.1 °C resolution) once a day starting on day 0. Light intensity was also measured using LI-COR LI-190 Quantum Sensor and LI-COR LI-1400 Datalogger for all floways (n=16) once a day starting on day 4 of the experiment.

Analysis protocols

To estimate the algal portion of the periphyton biomass, chl-a was extracted using 90% acetone (ACS grade) following the Environmental Sciences Section (ESS) method 150.1 (EPA 1991) and were calculated as chl-a, b, and c (uncorrected) and chl-a corrected for pheophytin based on equations described in Standard Methods (APHA 2005). Rather than filtering algae, I placed whole tiles with algae attached directly into 50-mL centrifuge tubes and filled each with 35 mL of 90% acetone. Whirl-paks were rinsed with small amount of the 90% acetone solution to remove algae residual remaining in the bags (total volume was always maintained at 35 mL).

For DM and AFDM analysis, periphyton was scrubbed from the tiles using a nylon bristled brush and filtered through pre-rinsed, pre-combusted, and pre-weighed GF/F filters (1.0 μm) following the ESS method 340.2 (EPA 1993). Samples were dried at 105 °C for a minimum of 12 h before weighing for DM and then were burned in a muffle oven at 550 °C for 30 min prior to the determination of ash mass. AFDM was estimated as the difference between DM and ash mass. To increase accuracy of the measurement, LG portable dehumidifier (QA114CBD) was used to remove moisture in the lab room during the analysis. Also, a small amount of W. A. Hammond Drierite was placed in the scale chamber and the holding bins to further maintain humidity under 20% at each weighing. Humidity was measured using Kestrel 4000 Pocket Weather Tracker.

Statistical analyses

Chl-a, DM, and AFDM were compared between treatment (with heaters) and control (without heaters) tiles using separate repeated measures analysis of variance (RM ANOVA) because the measurement of the dependent variable (chl-a, DM, or AFDM) was repeated. Date and treatment were used as independent variables for each analysis. Five sample dates were included in the analysis and yielded 80 replicates (i.e., 5 dates x 8 replicates x 2 treatments).

Water temperature and light intensity that were measured on the five sampling dates were also analyzed using RM ANOVA. Eight replicates within each treatment were analyzed for all sampling dates (n=5) yielding a total of 40 replicates/treatment.

All statistical analyses were conducted using IBM SPSS Statistics for Windows (21.0). Differences for all analysis were considered to be statistically significant if they exceeded the alpha of 0.05.

RESULTS

Temperature and light

The average control temperature was 21.2 ± 0.3 °C (mean ± 1 standard deviation) while the treatment floways temperatures were elevated an average of 1.7 ± 0.1 °C during the 20-day experiment (Table 7). Water temperature was significantly higher in the treatment group (RM ANOVA, $F_{1,14}$=2472.1, p<0.001). Daily mean temperature remained consistent in both treatments (CV for treatment was 0.47%; CV for control was 0.44%) over the course of the experiment.

Light intensity significantly changed over time (Figure 8, RM ANOVA, $F_{4,56}$=6.0, p<0.001); however, it did not differ between treatments (RM ANOVA, $F_{1,14}$=0.88, p=0.364). Also, there was no significant interaction between date and treatment (RM ANOVA, $F_{4,56}$=0.17, p=0.954).

Table 7. Mean water temperature (°C) in treatments (heated) and controls (non-heated) on sampling dates. Data are means ± standard deviations.

	Day 4	Day 8	Day 12	Day 16	Day 20
Control	21.0±0.1	21.0±0.1	21.1±0.2	21.1±0.1	21.1±0.2
Treatment	22.9±0.1	22.8±0.1	22.8±0.1	23.0±0.1	23.0±0.1

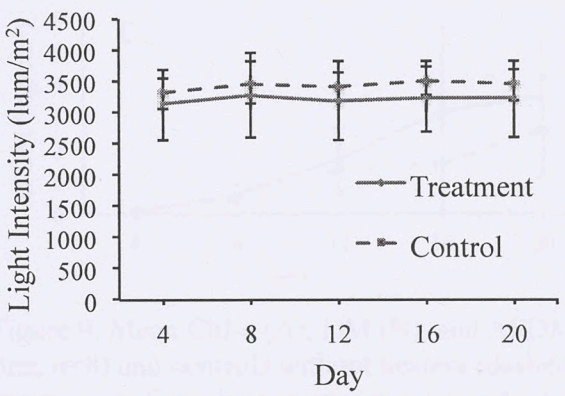

Figure 8. Mean light intensity in ATS floways treated with heaters (solid line) and controls without heaters (dashed line). Error bars represent ± 1 standard deviation.

Periphyton biomass

Chl-a (RM ANOVA, $F_{4,56}$=17.2, p<0.001), DM (RM ANOVA, $F_{4,56}$=17.2, p<0.001), and AFDM (RM ANOVA, $F_{4,56}$=17.7, p<0.001) all accumulated rapidly over the course of the 20-day experiment (Figure 9). Specifically, chl-a, DM and AFDM grew by 98%, 99%, and 97% in controls and 95%, 99%, and 98% in treatments, respectively, from day 4 to day 20 of the experiment. The change over time was similar between DM and AFDM, both of which appeared to increase linearly. Chl-a proliferated and peaked on or before day 16 of the experiment. However, elevated temperature (+1.7 °C above ambient) had no significant effect on chl-a (RM ANOVA, $F_{1,14}$=0.02, p=0.899), DM (RM ANOVA, $F_{1,14}$=2.9, p=0.111), or AFDM (RM ANOVA, $F_{1,14}$=3.2, p=0.093).

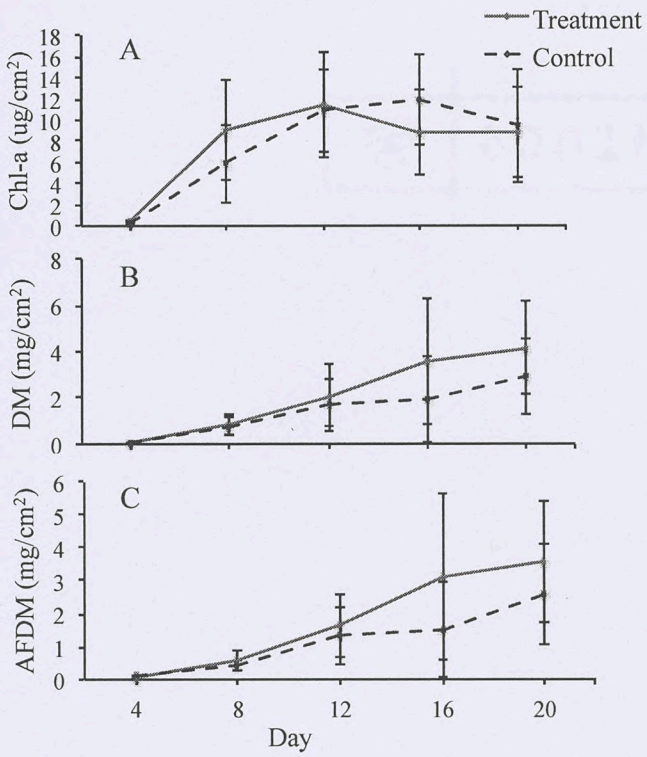

Figure 9. Mean Chl-a (A), DM (B), and AFDM (C) in ATS floways treated with heaters (solid line, n=8) and controls without heaters (dashed line, n=8) throughout the 20 day temperature experiment. Error bars represent ± 1 standard deviation.

DISCUSSION

Elevated water temperature (i.e. 1.7 °C above ambient) had no significant effect on periphyton biomass accrual in recirculating ATS. My hypothesis was not supported, since I hypothesized that higher temperatures would promote algal biomass accumulation. Previous publications have confirmed that periphyton biomass increases with elevated temperature. Tarkowska-Kukuryk & Mieczan (2012) found significant seasonal effects on periphyton biomass under eutrophic conditions, specifically, summer was 93% higher than winter (water temperature on average elevated by 15.7 °C). The cause of this effect was confounded because both light and temperature change seasonally. Mahdy *et al.* (2015), who observed 70% increase in periphyton biomass with an elevated temperature of 8.3 °C under eutrophic conditions.

There are a few possible explanations why the results of this study differed from these previous studies. Prior research examining temperature effects on periphyton were conducted in relatively low nutrient (TP ranging from 0.065 mg/L to 0.18 mg/L) environments. Because nutrient concentration in my study were more than ten times higher than these studies, it seems unlikely that nutrients limited periphyton responses to elevated temperature. In addition, their studies were conducted with a much larger temperature fluctuation. In my study, water temperature was increased only by 1.7 °C. The effect of warming on periphyton might be different at small temperature variations. Shurin *et al.* (2012) found that periphyton biomass declined with 3 °C temperature increase. Finally, previous experiments were conducted outdoors, receiving ambient sunlight for periphyton growth, while my experiment was conducted using artificial lights. Hansson (1992) reported nutrient and light availability were major factors regulating periphyton biomass accumulation. Similarly, Bowes *et al.* (2012) found that periphyton biomass was reduced by 30% when light intensity decreased by 40% and biomass

further declined with increased shading. Furthermore, Sanches *et al.* (2011) confirmed that periphyton biomass declined with decreasing light intensity, even in high nutrient environments. In my study, the average daytime light intensity ranged from 3,208 to 3,426 lum/m^2; however, the average daytime light intensity of sunlight is 15,700 lum/m^2 (Bowes *et al.* 2012), which was much higher than artificial lights. Therefore, light limitation may have limited periphyton growth and limited this study's capacity to detect temperature related effects.

In conclusion, my results indicate that a 1.7 °C temperature increase had no detectable effect on the growth of periphytic algae in laboratory, recirculating ATS. These findings suggest that the effectiveness of nutrient removal by ATS is unlikely to be strongly influenced by small changes in temperature, because the algal biomass is not affected by small temperature differences. My research examined periphyton responses to an elevated temperature of 1.7 °C over a short period; however, seasonal changes in temperature and light intensity may strongly affect periphyton biomass, which further affects the effectiveness of nutrient removal by ATS. Future studies need to examine how temperature and light interact to affect biomass accrual to gain a more comprehensive understanding of the performance of ATS across changing seasons and under future climate scenarios.

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APPENDIX A

Time Series Plot of Physical Parameters

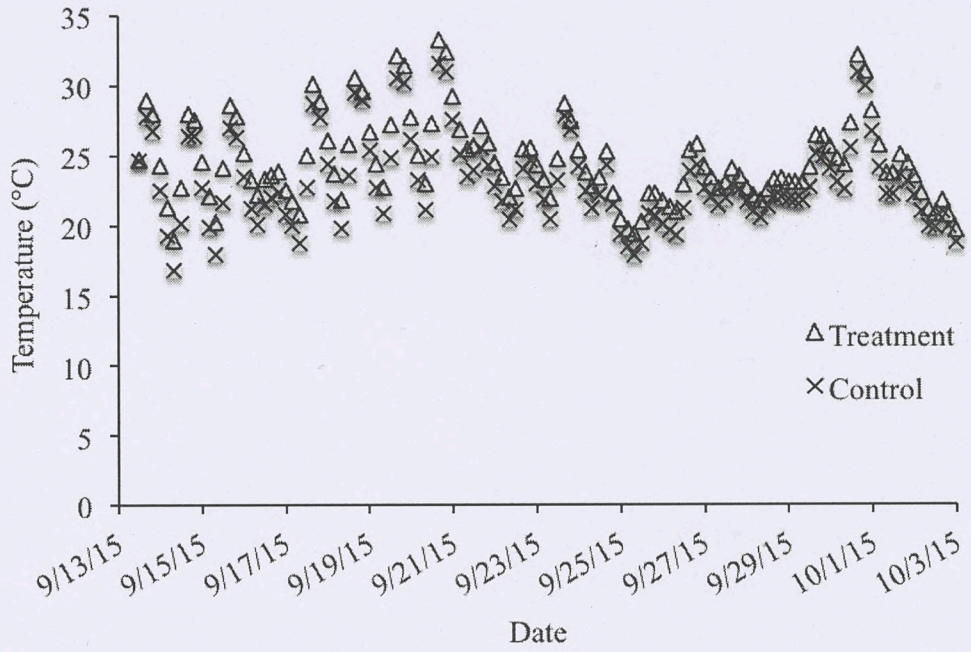

Figure 1. Water Temperature in *Bti* Treatments and Controls in Trial One

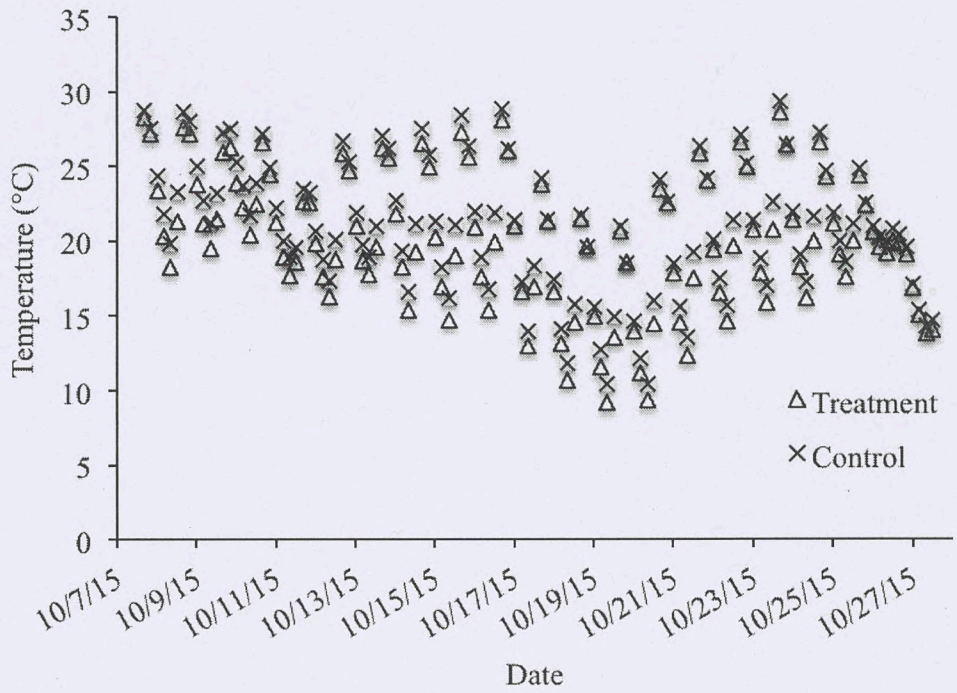

Figure 2. Water Temperature in *Bti* Treatments and Controls in Trial Two

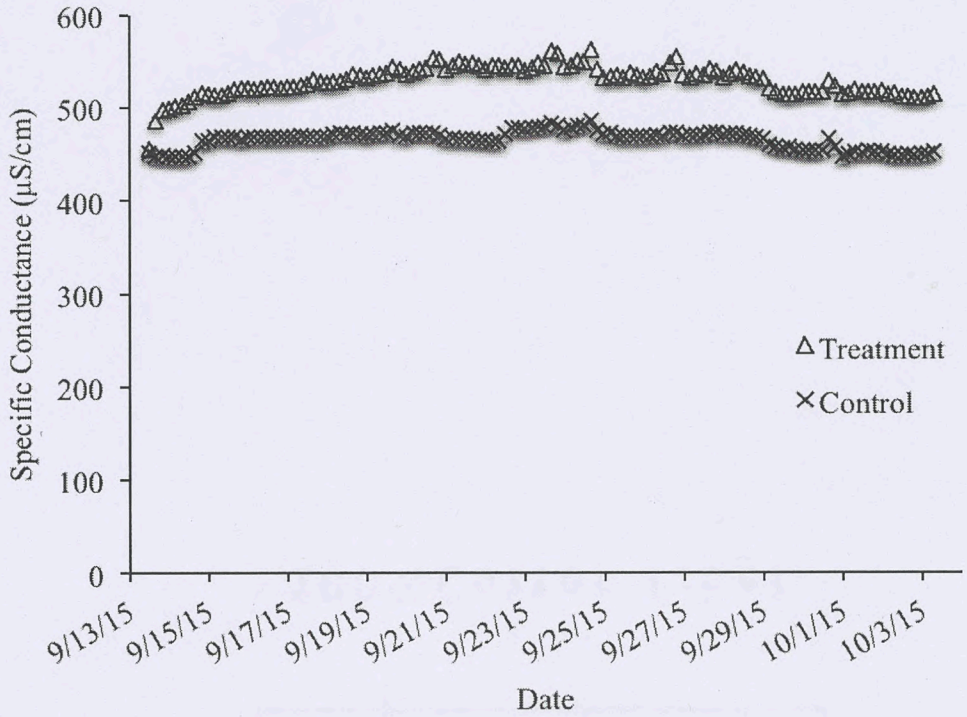

Figure 3. Specific Conductance in *Bti* Treatments and Controls in Trial One

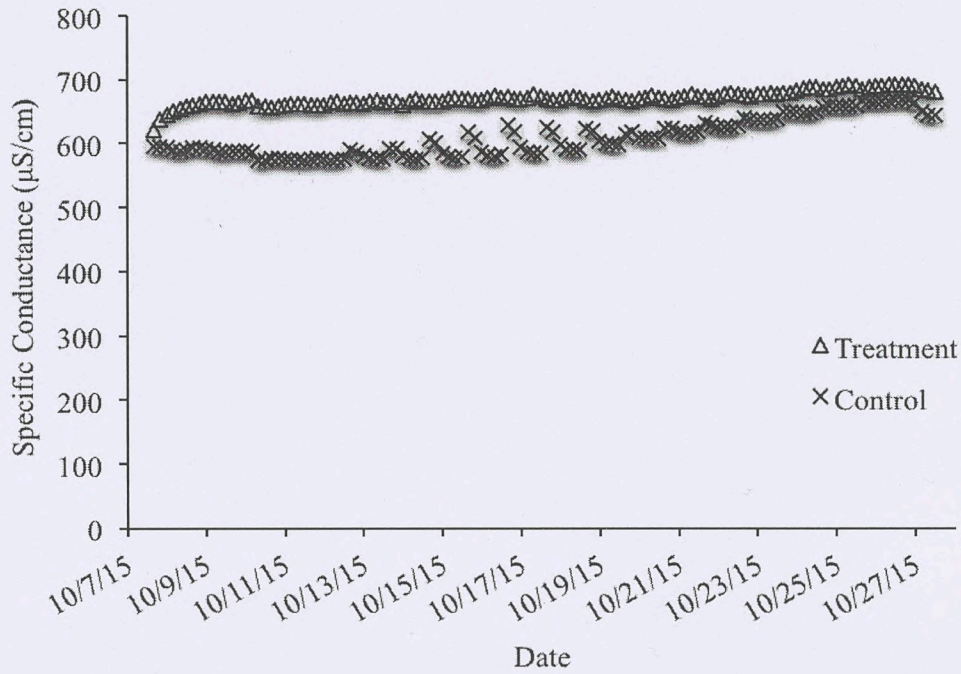

Figure 4. Specific Conductance in *Bti* Treatments and Controls in Trial Two

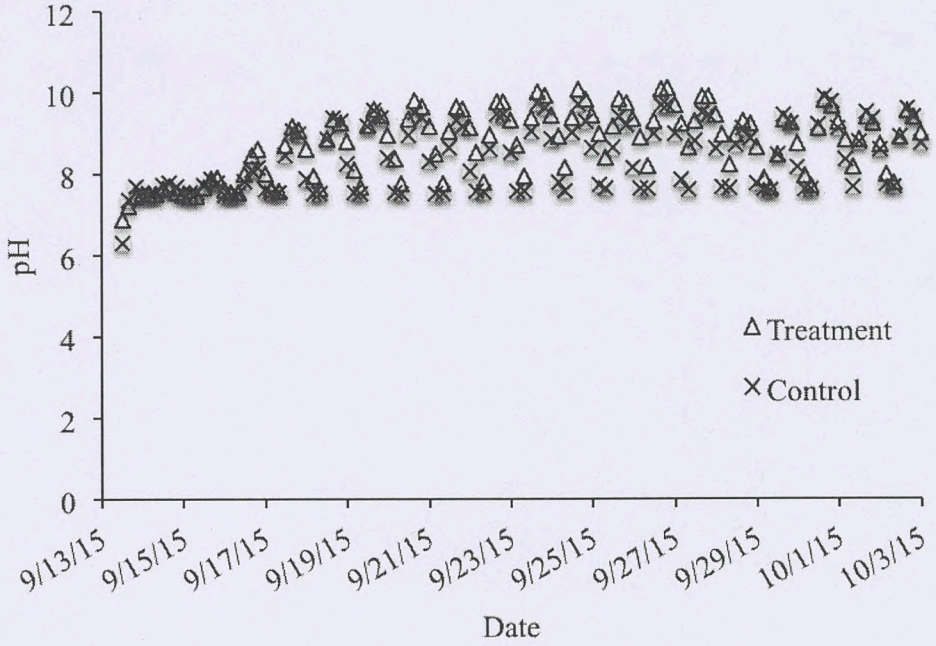

Figure 5. Water pH in *Bti* Treatments and Controls in Trial One

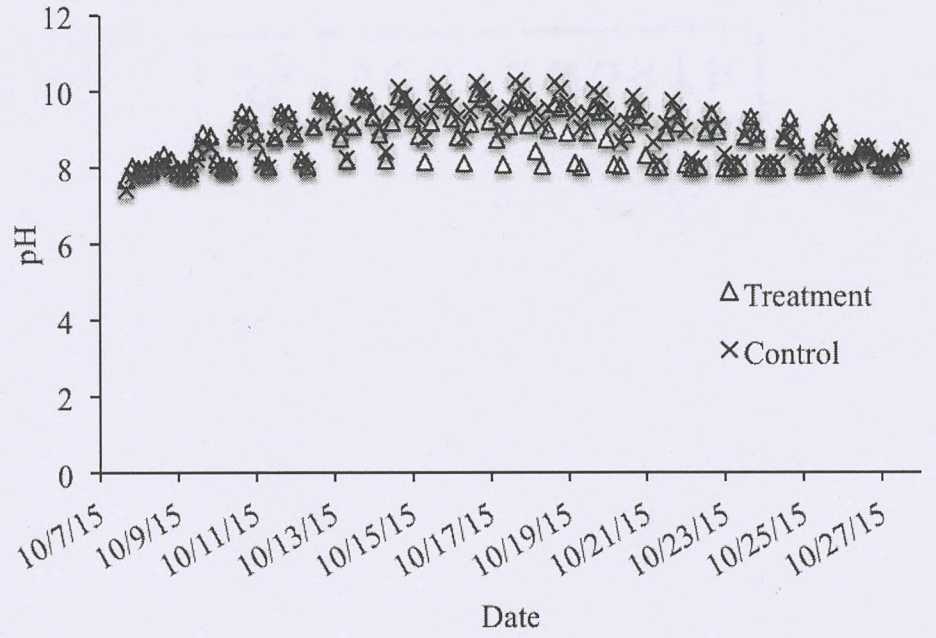

Figure 6. Water pH in *Bti* Treatments and Controls in Trial Two

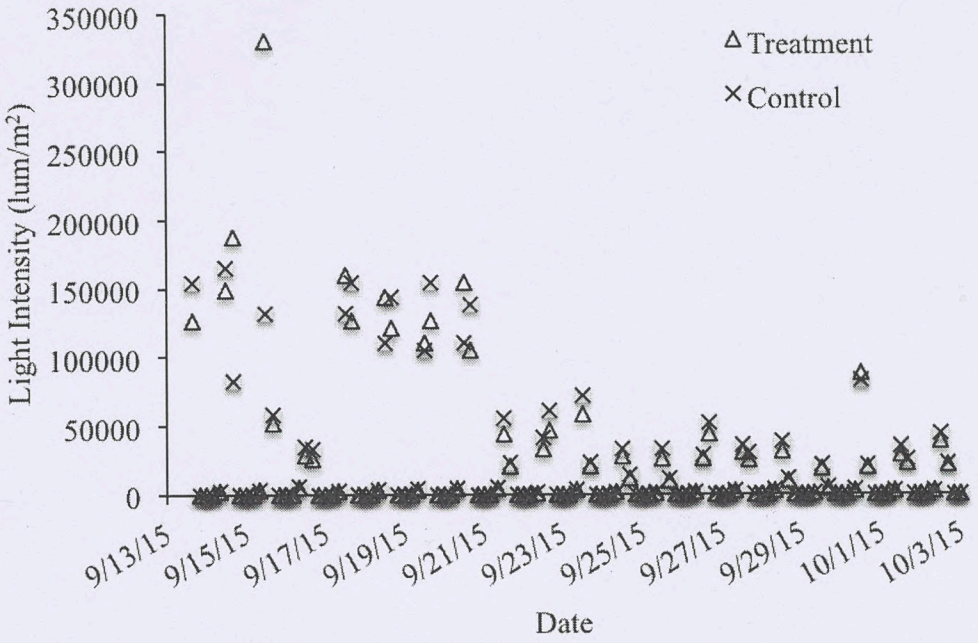

Figure 7. Floway Light Intensity in *Bti* Treatments and Controls in Trial One

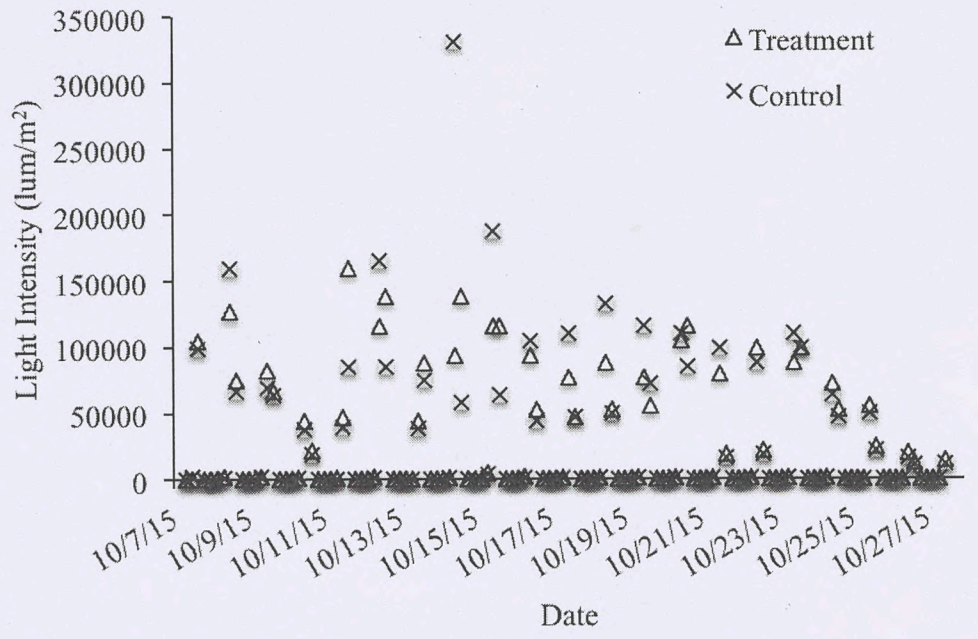

Figure 8. Floway Light Intensity in *Bti* Treatments and Controls in Trial Two

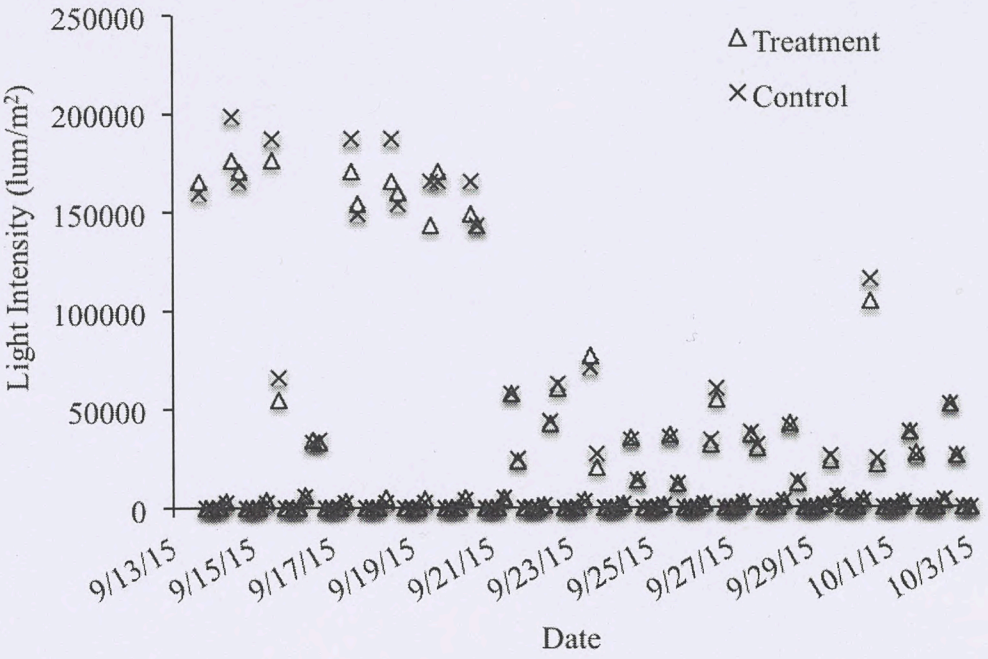

Figure 9. Outside Light Intensity in *Bti* Treatments and Controls in Trial One

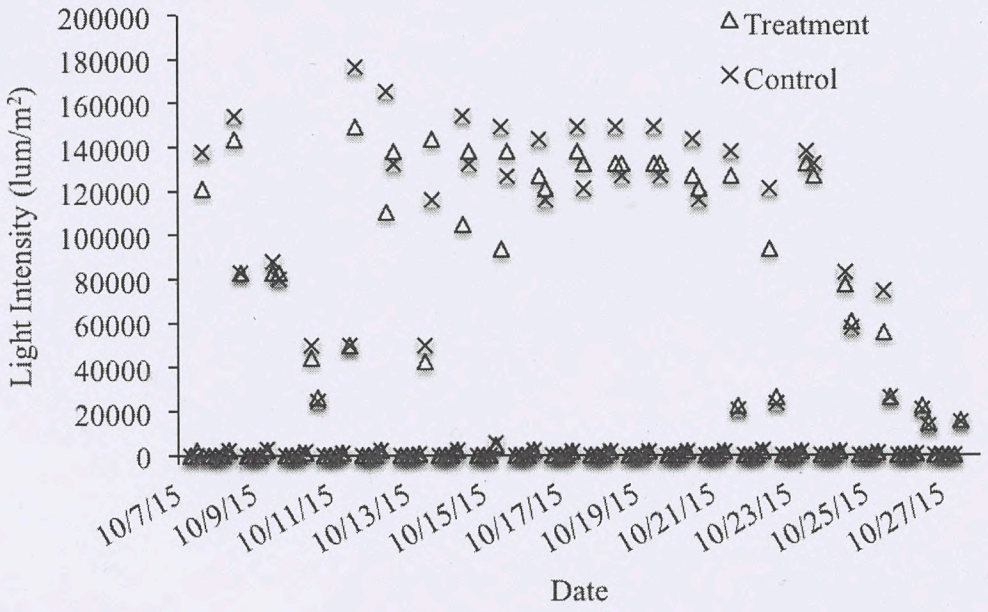

Figure 10. Outside Light Intensity in *Bti* Treatments and Controls in Trial Two

APPENDIX B

Midge Populations in ATS

Table 1. Midge Population Counts in *Bti* Treatments and Controls in Trial One

Date	Day	Treatment	Flume	Midge Count
9/17/2015	4	*Bti*	1	0
9/17/2015	4	*Bti*	2	0
9/17/2015	4	*Bti*	3	0
9/17/2015	4	*Bti*	4	0
9/17/2015	4	*Bti*	5	0
9/17/2015	4	*Bti*	6	0
9/17/2015	4	*Bti*	7	0
9/17/2015	4	*Bti*	8	0
9/17/2015	4	Control	1	1
9/17/2015	4	Control	2	11
9/17/2015	4	Control	3	5
9/17/2015	4	Control	4	4
9/17/2015	4	Control	5	5
9/17/2015	4	Control	6	4
9/17/2015	4	Control	7	3
9/17/2015	4	Control	8	5
9/21/2015	8	*Bti*	1	1
9/21/2015	8	*Bti*	2	1
9/21/2015	8	*Bti*	3	1
9/21/2015	8	*Bti*	4	1
9/21/2015	8	*Bti*	5	2
9/21/2015	8	*Bti*	6	0
9/21/2015	8	*Bti*	7	0
9/21/2015	8	*Bti*	8	0
9/21/2015	8	Control	1	11
9/21/2015	8	Control	2	9
9/21/2015	8	Control	3	15
9/21/2015	8	Control	4	6
9/21/2015	8	Control	5	21
9/21/2015	8	Control	6	12
9/21/2015	8	Control	7	20
9/21/2015	8	Control	8	14
9/25/2015	12	*Bti*	1	1
9/25/2015	12	*Bti*	2	3
9/25/2015	12	*Bti*	3	5
9/25/2015	12	*Bti*	4	1
9/25/2015	12	*Bti*	5	3
9/25/2015	12	*Bti*	6	3
9/25/2015	12	*Bti*	7	8

Date	Day	Treatment	Flume	Midge Count
9/25/2015	12	*Bti*	8	0
9/25/2015	12	Control	1	17
9/25/2015	12	Control	2	18
9/25/2015	12	Control	3	15
9/25/2015	12	Control	4	19
9/25/2015	12	Control	5	12
9/25/2015	12	Control	6	13
9/25/2015	12	Control	7	16
9/25/2015	12	Control	8	12
9/29/2015	16	*Bti*	1	7
9/29/2015	16	*Bti*	2	1
9/29/2015	16	*Bti*	3	4
9/29/2015	16	*Bti*	4	4
9/29/2015	16	*Bti*	5	1
9/29/2015	16	*Bti*	6	2
9/29/2015	16	*Bti*	7	2
9/29/2015	16	*Bti*	8	1
9/29/2015	16	Control	1	7
9/29/2015	16	Control	2	11
9/29/2015	16	Control	3	12
9/29/2015	16	Control	4	12
9/29/2015	16	Control	5	11
9/29/2015	16	Control	6	15
9/29/2015	16	Control	7	9
9/29/2015	16	Control	8	10
10/3/2015	20	*Bti*	1	1
10/3/2015	20	*Bti*	2	1
10/3/2015	20	*Bti*	3	5
10/3/2015	20	*Bti*	4	0
10/3/2015	20	*Bti*	5	0
10/3/2015	20	*Bti*	6	2
10/3/2015	20	*Bti*	7	3
10/3/2015	20	*Bti*	8	4
10/3/2015	20	Control	1	8
10/3/2015	20	Control	2	12
10/3/2015	20	Control	3	14
10/3/2015	20	Control	4	7
10/3/2015	20	Control	5	11
10/3/2015	20	Control	6	7
10/3/2015	20	Control	7	17
10/3/2015	20	Control	8	15

Table 2. Midge Population Counts in *Bti* Treatments and Controls in Trial Two

Date	Day	Treatment	Flume	Midge Count
10/11/2015	4	*Bti*	1	1
10/11/2015	4	*Bti*	2	0
10/11/2015	4	*Bti*	3	0
10/11/2015	4	*Bti*	4	0
10/11/2015	4	*Bti*	5	0
10/11/2015	4	*Bti*	6	0
10/11/2015	4	*Bti*	7	0
10/11/2015	4	*Bti*	8	0
10/11/2015	4	Control	1	0
10/11/2015	4	Control	2	0
10/11/2015	4	Control	3	0
10/11/2015	4	Control	4	1
10/11/2015	4	Control	5	1
10/11/2015	4	Control	6	0
10/11/2015	4	Control	7	0
10/11/2015	4	Control	8	0
10/15/2015	8	*Bti*	1	0
10/15/2015	8	*Bti*	2	0
10/15/2015	8	*Bti*	3	0
10/15/2015	8	*Bti*	4	0
10/15/2015	8	*Bti*	5	0
10/15/2015	8	*Bti*	6	0
10/15/2015	8	*Bti*	7	0
10/15/2015	8	*Bti*	8	0
10/15/2015	8	Control	1	1
10/15/2015	8	Control	2	1
10/15/2015	8	Control	3	1
10/15/2015	8	Control	4	0
10/15/2015	8	Control	5	1
10/15/2015	8	Control	6	2
10/15/2015	8	Control	7	1
10/15/2015	8	Control	8	2
10/19/2015	12	*Bti*	1	0
10/19/2015	12	*Bti*	2	0
10/19/2015	12	*Bti*	3	0
10/19/2015	12	*Bti*	4	0
10/19/2015	12	*Bti*	5	0
10/19/2015	12	*Bti*	6	0
10/19/2015	12	*Bti*	7	0
10/19/2015	12	*Bti*	8	0
10/19/2015	12	Control	1	0
10/19/2015	12	Control	2	1
10/19/2015	12	Control	3	0

Date	Day	Treatment	Flume	Midge Count
10/19/2015	12	Control	4	0
10/19/2015	12	Control	5	1
10/19/2015	12	Control	6	0
10/19/2015	12	Control	7	0
10/19/2015	12	Control	8	0
10/23/2015	16	*Bti*	1	0
10/23/2015	16	*Bti*	2	1
10/23/2015	16	*Bti*	3	0
10/23/2015	16	*Bti*	4	0
10/23/2015	16	*Bti*	5	0
10/23/2015	16	*Bti*	6	0
10/23/2015	16	*Bti*	7	0
10/23/2015	16	*Bti*	8	0
10/23/2015	16	Control	1	4
10/23/2015	16	Control	2	3
10/23/2015	16	Control	3	1
10/23/2015	16	Control	4	3
10/23/2015	16	Control	5	6
10/23/2015	16	Control	6	3
10/23/2015	16	Control	7	8
10/23/2015	16	Control	8	3
10/27/2015	20	*Bti*	1	0
10/27/2015	20	*Bti*	2	0
10/27/2015	20	*Bti*	3	1
10/27/2015	20	*Bti*	4	0
10/27/2015	20	*Bti*	5	1
10/27/2015	20	*Bti*	6	0
10/27/2015	20	*Bti*	7	0
10/27/2015	20	*Bti*	8	0
10/27/2015	20	Control	1	3
10/27/2015	20	Control	2	5
10/27/2015	20	Control	3	1
10/27/2015	20	Control	4	0
10/27/2015	20	Control	5	0
10/27/2015	20	Control	6	2
10/27/2015	20	Control	7	2
10/27/2015	20	Control	8	2

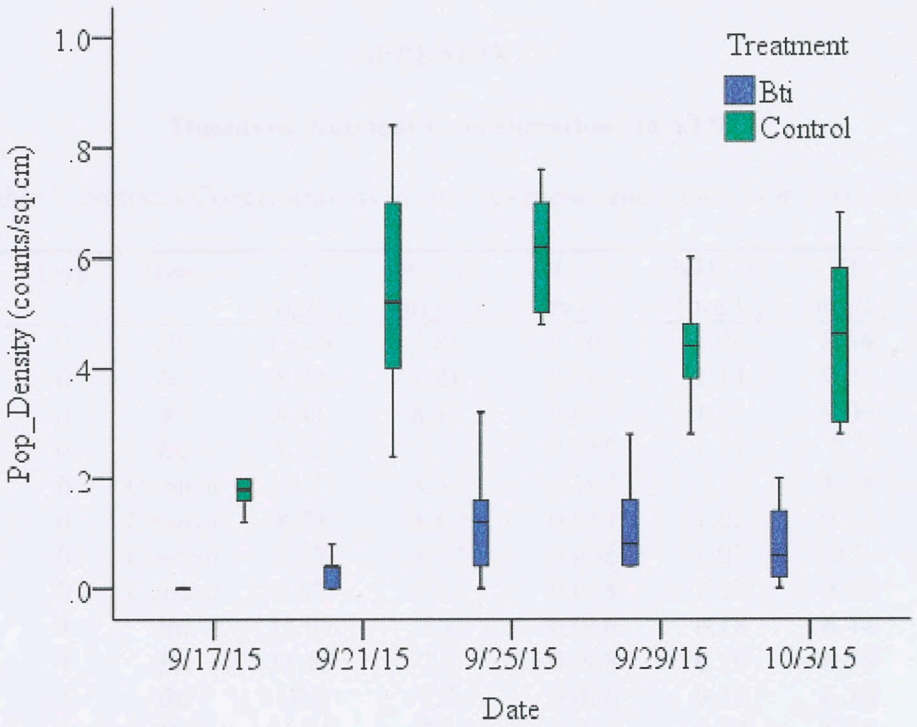

Figure 1. *Bti* treatment effect on midge population density in trial one

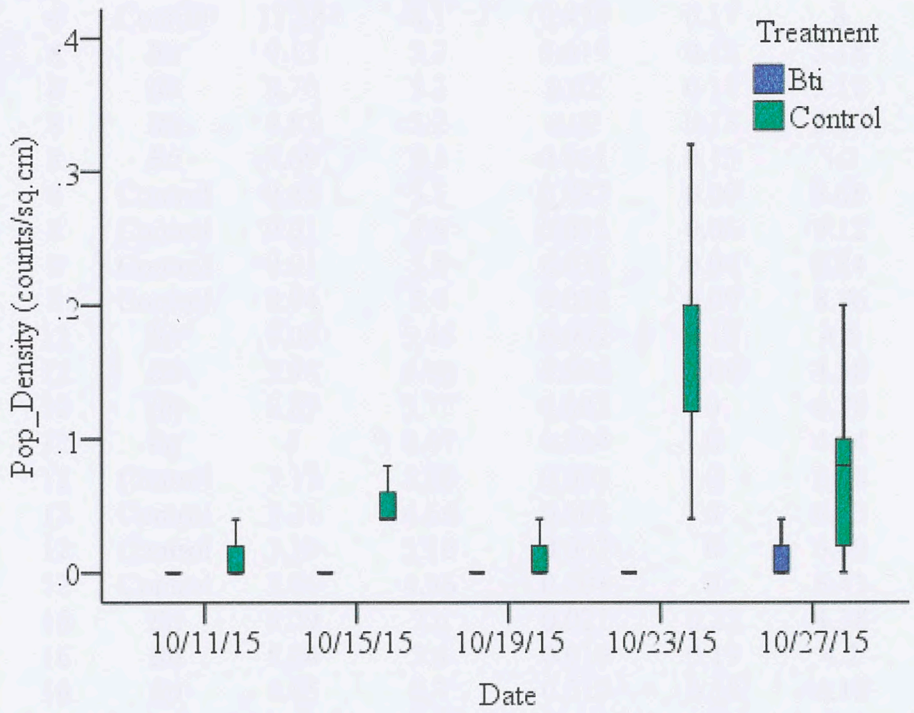

Figure 2. *Bti* treatment effect on midge population density in trial two

APPENDIX C

Dissolved Nutrient Concentrations in ATS

Table 1. Nutrient Concentrations in *Bti* Treatments and Controls in Trial One

Date	Day	Treat.	TN (mg/L)	NO_3^- -N (mg/L)	NO_2^- -N (mg/L)	NH_3 -N (mg/L)	TP (mg/L)	PO_4^{3-} -P (mg/L)
9/13/2015	0	*Bti*	13.48	7.81	0.108	1.06	8.56	5.72
9/13/2015	0	*Bti*	8.22	8.21	0.063	1.14	9.06	5.66
9/13/2015	0	*Bti*	8.41	8.11	0.061	1.11	9.06	5.58
9/13/2015	0	*Bti*	8.35	7.9	0.059	1.11	9.3	5.62
9/13/2015	0	Control	10.77	8.52	0.107	1.1	8.68	5.56
9/13/2015	0	Control	8.74	8.62	0.057	1.02	9.53	5.58
9/13/2015	0	Control	8.17	8.32	0.056	1.07	9.3	5.54
9/13/2015	0	Control	8.67	8.52	0.058	1.07	9.65	5.42
9/17/2015	4	*Bti*	11.97	7.4	0.066	0.18	6.44	5.44
9/17/2015	4	*Bti*	11.59	7.1	0.068	0.19	6.58	5.36
9/17/2015	4	*Bti*	10.9	7.3	0.066	0.18	6.36	5.36
9/17/2015	4	*Bti*	11.21	7.1	0.063	0.17	6.16	5.2
9/17/2015	4	Control	11.65	8	0.488	0.18	7.52	6.12
9/17/2015	4	Control	11.97	7.8	0.508	0.19	8.28	6
9/17/2015	4	Control	11.4	8.3	0.486	0.17	8.12	6.2
9/17/2015	4	Control	11.28	8.1	0.478	0.17	8	6.12
9/21/2015	8	*Bti*	9.13	5.2	0.019	0.15	3.18	3.1
9/21/2015	8	*Bti*	8.76	5.3	0.02	0.14	3.12	2.92
9/21/2015	8	*Bti*	9.83	5.2	0.02	0.13	3.17	2.94
9/21/2015	8	*Bti*	8.69	5.1	0.021	0.13	3.2	2.9
9/21/2015	8	Control	9.45	5.1	0.033	0.09	8.68	7.68
9/21/2015	8	Control	9.01	5.6	0.031	0.06	9.12	7.8
9/21/2015	8	Control	9.01	5.8	0.031	0.04	8.84	7.76
9/21/2015	8	Control	8.94	5.4	0.032	0.09	8.76	8.36
9/25/2015	12	*Bti*	4.06	5.45	0.002	0.16	4.6	6.12
9/25/2015	12	*Bti*	2.94	4.56	0.002	0.09	4.58	5.96
9/25/2015	12	*Bti*	2.87	3.77	0.003	0	4.58	5.96
9/25/2015	12	*Bti*	3	4.07	0.004	0	4.54	6.04
9/25/2015	12	Control	3.12	5.06	0.003	0	6.58	8.44
9/25/2015	12	Control	3.31	4.66	0.003	0	6.43	7.92
9/25/2015	12	Control	3.19	5.16	0.003	0	6.49	8.36
9/25/2015	12	Control	3.06	4.86	0.004	0	6.43	7.76
9/29/2015	16	*Bti*	5.29	2.6	0.021	0.22	4.38	4
9/29/2015	16	*Bti*	5.04	2.4	0.019	0.19	4.2	4.02
9/29/2015	16	*Bti*	4.85	2.7	0.017	0.18	4.18	3.92
9/29/2015	16	*Bti*	4.54	2.7	0.02	0.15	4.3	4.02
9/29/2015	16	Control	5.73	3	0.015	0.14	5.76	7.08
9/29/2015	16	Control	5.98	3.2	0.015	0.12	5.82	7.28

Date	Day	Treat.	TN (mg/L)	NO_3^- -N (mg/L)	NO_2^- -N (mg/L)	NH_3 -N (mg/L)	TP (mg/L)	PO_4^{3-} -P (mg/L)
9/29/2015	16	Control	5.86	4.4	0.016	0.1	5.82	7.04
9/29/2015	16	Control	5.86	3.1	0.015	0.06	5.88	7
10/3/2015	20	*Bti*	0	1.6	0.027	0.33	3	2.74
10/3/2015	20	*Bti*	0	1.5	0.026	0.26	2.16	2.52
10/3/2015	20	*Bti*	2.96	1.4	0.03	0.27	2.42	2.42
10/3/2015	20	*Bti*	0.38	1.4	0.029	0.24	2.28	2.56
10/3/2015	20	Control	0.063	2.2	0.009	0.23	2.8	3.26
10/3/2015	20	Control	0	2.2	0.008	0.15	3	3.32
10/3/2015	20	Control	0.378	2.3	0.005	0.08	2.7	3.32
10/3/2015	20	Control	0.63	2.4	0.006	0.03	2.8	3.34

Table 2. Nutrient Concentrations in *Bti* Treatments and Controls in Trial Two

Date	Day	Treat.	TN (mg/L)	NO_3^- -N (mg/L)	NO_2^- -N (mg/L)	NH_3 -N (mg/L)	TP (mg/L)	PO_4^{3-} -P (mg/L)
10/7/2015	0	*Bti*	11.33	6.5	0.139	1.67	3.25	2.8
10/7/2015	0	*Bti*	11.52	7.2	0.141	1.66	3.35	2.88
10/7/2015	0	*Bti*	12.16	6.7	0.143	1.61	3.35	2.7
10/7/2015	0	*Bti*	12.1	6.6	0.142	1.61	3.27	2.78
10/7/2015	0	Control	11.78	6.3	0.127	2.08	3.45	3.18
10/7/2015	0	Control	11.46	6.4	0.125	2.09	3.3	2.92
10/7/2015	0	Control	10.83	5.8	0.126	2.06	3.43	3.02
10/7/2015	0	Control	11.53	6.1	0.125	2	3.36	2.86
10/11/2015	4	*Bti*	9.98	6.3	0.008	0.11	2.67	2.24
10/11/2015	4	*Bti*	10.69	6.5	0.01	0.05	2.68	2.22
10/11/2015	4	*Bti*	10.62	7	0.011	0.02	2.98	2.18
10/11/2015	4	*Bti*	10.88	6.3	0.01	0	2.67	2.2
10/11/2015	4	Control	9.45	6.8	0.015	0.2	3.21	2.7
10/11/2015	4	Control	9.89	6.5	0.015	0.19	3.28	2.68
10/11/2015	4	Control	10.27	7.5	0.015	0.17	3.11	2.66
10/11/2015	4	Control	10.9	7.4	0.014	0.12	3.04	2.58
10/15/2015	8	*Bti*	12.8	2.5	0	0.15	2.23	0.07
10/15/2015	8	*Bti*	10.69	2.5	0.002	0.38	2.15	0.08
10/15/2015	8	*Bti*	19.33	2.7	0.006	0.17	3.24	0.05
10/15/2015	8	*Bti*	13.57	2.7	0	0.17	3.55	0.07
10/15/2015	8	Control	4.66	2.8	0.005	0.21	0.81	0.09
10/15/2015	8	Control	4.03	2.9	0.005	0.2	0.58	0.07
10/15/2015	8	Control	4.16	2.4	0.007	0.19	0.81	0.07
10/15/2015	8	Control	4.03	2.7	0.01	0.15	0.89	0.07
10/19/2015	12	*Bti*	22.98	0.1	0	0.14	1.61	0.11
10/19/2015	12	*Bti*	25.54	0.1	0	0	6.24	0.08
10/19/2015	12	*Bti*	7.74	0.1	0	0.04	1.93	0.07
10/19/2015	12	*Bti*	16.58	0.1	0.002	0.02	3.74	0.08

Date	Day	Treat.	TN (mg/L)	NO_3^- -N (mg/L)	NO_2^- -N (mg/L)	NH_3 -N (mg/L)	TP (mg/L)	PO_4^{3-} -P (mg/L)
10/19/2015	12	Control	1.7	0.2	0	0.21	0.4	0.07
10/19/2015	12	Control	0	0.2	0.001	0.2	0.45	0.05
10/19/2015	12	Control	0.44	0.2	0	0.18	0.59	0.07
10/19/2015	12	Control	1.26	0.2	0	0.15	0.56	0.05
10/23/2015	16	*Bti*	0	0	0	0.37	1.03	0
10/23/2015	16	*Bti*	0.32	0	0.011	0.41	1.02	0.04
10/23/2015	16	*Bti*	0.64	0	0	0.26	1.52	0.02
10/23/2015	16	*Bti*	1.73	0	0.003	0.26	1.01	0.06
10/23/2015	16	Control	0.06	0	0.002	0.38	1	0.05
10/23/2015	16	Control	2.46	0	0	0.35	0.79	0
10/23/2015	16	Control	2.39	0	0	0.35	0.76	0.01
10/23/2015	16	Control	0.25	0	0	0.27	0.8	0.05
10/27/2015	20	*Bti*	2.43	0.1	0.005	0.28	1.59	0
10/27/2015	20	*Bti*	1.02	0.1	0	0.51	1.21	0.05
10/27/2015	20	*Bti*	4.22	0.1	0	0.15	1.15	0.03
10/27/2015	20	*Bti*	0.45	0	0	0.17	0.54	0.08
10/27/2015	20	Control	0.69	0.2	0	0.31	0.81	0.07
10/27/2015	20	Control	1.95	0.2	0	0.34	0.72	0.03
10/27/2015	20	Control	1.7	0.1	0	0.33	0.77	0.01
10/27/2015	20	Control	0.19	0.2	0	0.23	0.83	0.04

APPENDIX D

Cellular Concentrations of Nutrient in Algae

Table 1. Algal Tissue Nutrients in *Bti* Treatments and Controls in Trial One

Date	Day	Treatment	Flume	TN (mg/g)	TP (mg/g)
9/17/2015	4	*Bti*	1	11.57	5.24
9/17/2015	4	*Bti*	2	8.14	4.02
9/17/2015	4	*Bti*	3	8.5	4.46
9/17/2015	4	*Bti*	4	9.4	4.38
9/17/2015	4	*Bti*	5	9.04	3.86
9/17/2015	4	*Bti*	6	10.12	4.28
9/17/2015	4	*Bti*	7	10.31	4.33
9/17/2015	4	*Bti*	8	11.75	4.46
9/17/2015	4	Control	1	11.21	4.07
9/17/2015	4	Control	2	11.21	4.36
9/17/2015	4	Control	3	9.76	4.43
9/17/2015	4	Control	4	10.12	3.6
9/17/2015	4	Control	5	11.39	4.17
9/17/2015	4	Control	6	10.85	4.46
9/17/2015	4	Control	7	9.22	4.25
9/17/2015	4	Control	8	10.31	3.63
9/21/2015	8	*Bti*	1	7.59	4.82
9/21/2015	8	*Bti*	2	7.77	4.33
9/21/2015	8	*Bti*	3	9.58	4.56
9/21/2015	8	*Bti*	4	11.21	4.67
9/21/2015	8	*Bti*	5	11.21	4.46
9/21/2015	8	*Bti*	6	10.67	4.8
9/21/2015	8	*Bti*	7	9.58	4.43
9/21/2015	8	*Bti*	8	10.12	4.43
9/21/2015	8	Control	1	10.31	4.54
9/21/2015	8	Control	2	10.49	4.28
9/21/2015	8	Control	3	8.5	4.36
9/21/2015	8	Control	4	9.4	4.25
9/21/2015	8	Control	5	9.76	4.69
9/21/2015	8	Control	6	10.49	4.12
9/21/2015	8	Control	7	8.86	4.36
9/21/2015	8	Control	8	12.11	3.86
9/25/2015	12	*Bti*	1	10.85	3.08
9/25/2015	12	*Bti*	2	10.85	2.82
9/25/2015	12	*Bti*	3	12.11	3.86
9/25/2015	12	*Bti*	4	9.04	5.06
9/25/2015	12	*Bti*	5	11.57	4.02
9/25/2015	12	*Bti*	6	10.49	4.17
9/25/2015	12	*Bti*	7	10.12	3.65

Date	Day	Treatment	Flume	TN (mg/g)	TP (mg/g)
9/25/2015	12	*Bti*	8	9.76	5.63
9/25/2015	12	Control	1	13.02	2.89
9/25/2015	12	Control	2	12.66	2.61
9/25/2015	12	Control	3	12.29	3.13
9/25/2015	12	Control	4	10.67	2.89
9/25/2015	12	Control	5	10.85	2.95
9/25/2015	12	Control	6	10.12	3.08
9/25/2015	12	Control	7	11.03	3.16
9/25/2015	12	Control	8	9.58	4.28
9/29/2015	16	*Bti*	1	12.66	1.47
9/29/2015	16	*Bti*	2	14.1	1.68
9/29/2015	16	*Bti*	3	12.84	1.41
9/29/2015	16	*Bti*	4	13.56	1.6
9/29/2015	16	*Bti*	5	15.14	1.16
9/29/2015	16	*Bti*	6	8.32	1.21
9/29/2015	16	*Bti*	7	11.39	1.29
9/29/2015	16	*Bti*	8	10.76	2.09
9/29/2015	16	Control	1	6.51	2.24
9/29/2015	16	Control	2	9.04	3.52
9/29/2015	16	Control	3	8.23	2.24
9/29/2015	16	Control	4	6.87	2.78
9/29/2015	16	Control	5	8.05	3.22
9/29/2015	16	Control	6	7.68	3.63
9/29/2015	16	Control	7	7.23	4.33
9/29/2015	16	Control	8	7.77	3.21
10/3/2015	20	*Bti*	1	8.95	2.24
10/3/2015	20	*Bti*	2	8.41	1.42
10/3/2015	20	*Bti*	3	7.77	1.41
10/3/2015	20	*Bti*	4	8.23	1.34
10/3/2015	20	*Bti*	5	8.68	1.79
10/3/2015	20	*Bti*	6	6.87	2.39
10/3/2015	20	*Bti*	7	7.59	1.47
10/3/2015	20	*Bti*	8	6.69	1.5
10/3/2015	20	Control	1	7.5	2.39
10/3/2015	20	Control	2	7.59	2.3
10/3/2015	20	Control	3	6.96	2.2
10/3/2015	20	Control	4	8.68	2.1
10/3/2015	20	Control	5	6.69	2.57
10/3/2015	20	Control	6	7.05	2.83
10/3/2015	20	Control	7	4.16	3.63
10/3/2015	20	Control	8	3.89	3.47

Table 2. Algal Tissue Nutrients in *Bti* Treatments and Controls in Trial Two

Date	Day	Treatment	Flume	TN (mg/g)	TP (mg/g)
10/11/2015	4	*Bti*	1	6.51	3.96
10/11/2015	4	*Bti*	2	5.15	4.69
10/11/2015	4	*Bti*	3	6.33	4.67
10/11/2015	4	*Bti*	4	6.06	4.51
10/11/2015	4	*Bti*	5	6.87	3.6
10/11/2015	4	*Bti*	6	6.42	4.9
10/11/2015	4	*Bti*	7	6.87	4.93
10/11/2015	4	*Bti*	8	6.78	3.63
10/11/2015	4	Control	1	6.69	3.65
10/11/2015	4	Control	2	6.6	5.06
10/11/2015	4	Control	3	3.34	4.36
10/11/2015	4	Control	4	5.7	4.17
10/11/2015	4	Control	5	6.69	4.3
10/11/2015	4	Control	6	6.6	4.9
10/11/2015	4	Control	7	5.42	3.78
10/11/2015	4	Control	8	6.6	3.83
10/15/2015	8	*Bti*	1	7.23	2.5
10/15/2015	8	*Bti*	2	7.05	2.48
10/15/2015	8	*Bti*	3	5.79	2.87
10/15/2015	8	*Bti*	4	6.69	2.35
10/15/2015	8	*Bti*	5	8.5	2.37
10/15/2015	8	*Bti*	6	7.77	1.85
10/15/2015	8	*Bti*	7	7.05	2.45
10/15/2015	8	*Bti*	8	7.59	2.24
10/15/2015	8	Control	1	6.42	3.86
10/15/2015	8	Control	2	6.06	4.36
10/15/2015	8	Control	3	6.51	4.15
10/15/2015	8	Control	4	6.87	3.68
10/15/2015	8	Control	5	6.69	3.63
10/15/2015	8	Control	6	6.33	4.9
10/15/2015	8	Control	7	7.41	4.22
10/15/2015	8	Control	8	6.33	3.44
10/19/2015	12	*Bti*	1	6.15	2.24
10/19/2015	12	*Bti*	2	6.51	1.54
10/19/2015	12	*Bti*	3	6.51	2.24
10/19/2015	12	*Bti*	4	6.33	3.03
10/19/2015	12	*Bti*	5	6.33	1.88
10/19/2015	12	*Bti*	6	7.41	2.09
10/19/2015	12	*Bti*	7	7.05	2.32
10/19/2015	12	*Bti*	8	6.51	2.71
10/19/2015	12	Control	1	5.24	3.57
10/19/2015	12	Control	2	5.42	3.47
10/19/2015	12	Control	3	4.52	3.52

Date	Day	Treatment	Flume	TN (mg/g)	TP (mg/g)
10/19/2015	12	Control	4	6.33	3.49
10/19/2015	12	Control	5	5.42	3.83
10/19/2015	12	Control	6	5.79	3.42
10/19/2015	12	Control	7	4.52	4.46
10/19/2015	12	Control	8	4.88	2.97
10/23/2015	16	*Bti*	1	5.79	1.98
10/23/2015	16	*Bti*	2	5.97	1.64
10/23/2015	16	*Bti*	3	7.05	1.96
10/23/2015	16	*Bti*	4	6.15	2.43
10/23/2015	16	*Bti*	5	7.05	2.11
10/23/2015	16	*Bti*	6	6.69	2.97
10/23/2015	16	*Bti*	7	6.69	2.09
10/23/2015	16	*Bti*	8	6.15	1.85
10/23/2015	16	Control	1	7.59	1.8
10/23/2015	16	Control	2	5.79	2.97
10/23/2015	16	Control	3	6.51	2.06
10/23/2015	16	Control	4	7.05	1.75
10/23/2015	16	Control	5	7.23	1.51
10/23/2015	16	Control	6	6.69	2.61
10/23/2015	16	Control	7	4.88	2.22
10/23/2015	16	Control	8	6.15	2.32
10/27/2015	20	*Bti*	1	6.51	2.35
10/27/2015	20	*Bti*	2	6.33	2.19
10/27/2015	20	*Bti*	3	7.05	1.51
10/27/2015	20	*Bti*	4	5.79	2.5
10/27/2015	20	*Bti*	5	6.51	1.9
10/27/2015	20	*Bti*	6	6.87	2.03
10/27/2015	20	*Bti*	7	6.33	2.24
10/27/2015	20	*Bti*	8	6.51	1.85
10/27/2015	20	Control	1	6.51	2.43
10/27/2015	20	Control	2	6.69	1.77
10/27/2015	20	Control	3	5.42	2.19
10/27/2015	20	Control	4	6.15	1.88
10/27/2015	20	Control	5	7.41	1.8
10/27/2015	20	Control	6	6.15	2.61
10/27/2015	20	Control	7	7.77	1.46
10/27/2015	20	Control	8	7.77	1.59

APPENDIX E

Periphyton Biomass in *Bti* Treatments and Controls in ATS

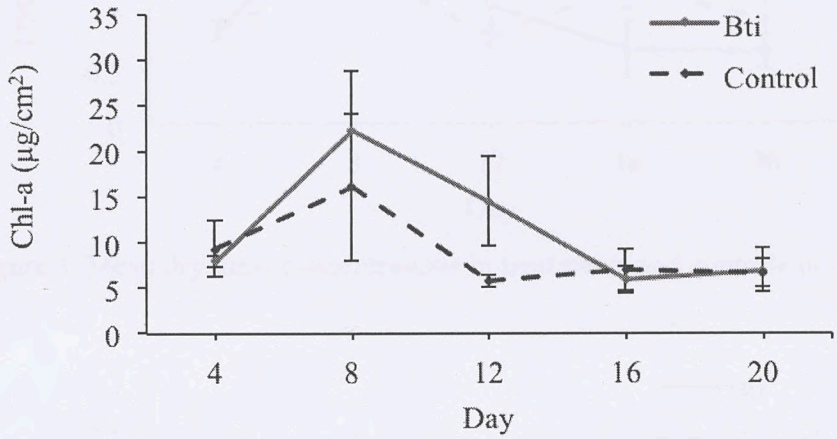

Figure 1. Mean chl-a concentrations in treatments and controls in trial one

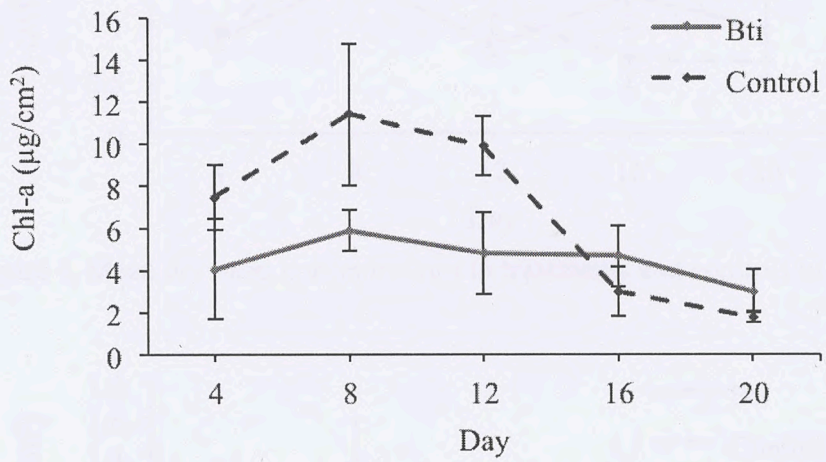

Figure 2. Mean chl-a concentrations in treatments and controls in trial two

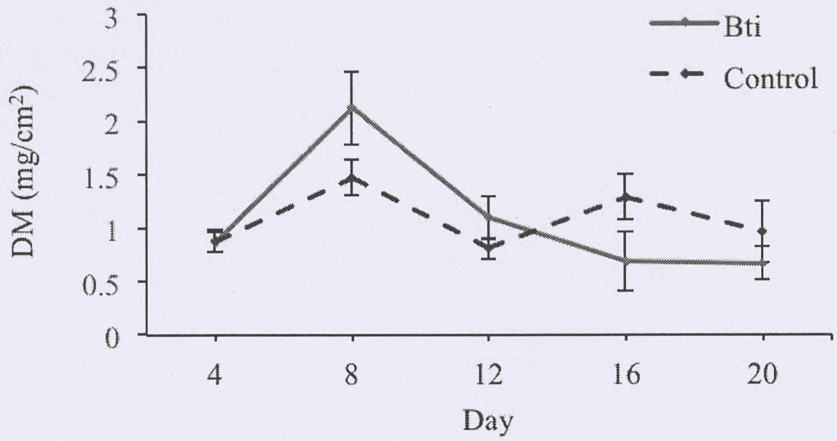

Figure 3. Mean dry mass concentrations in treatments and controls in trial one

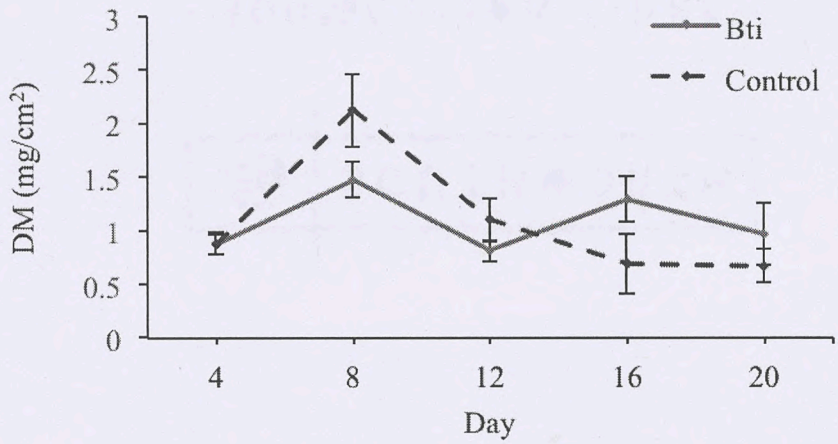

Figure 4. Mean dry mass concentrations in treatments and controls in trial two

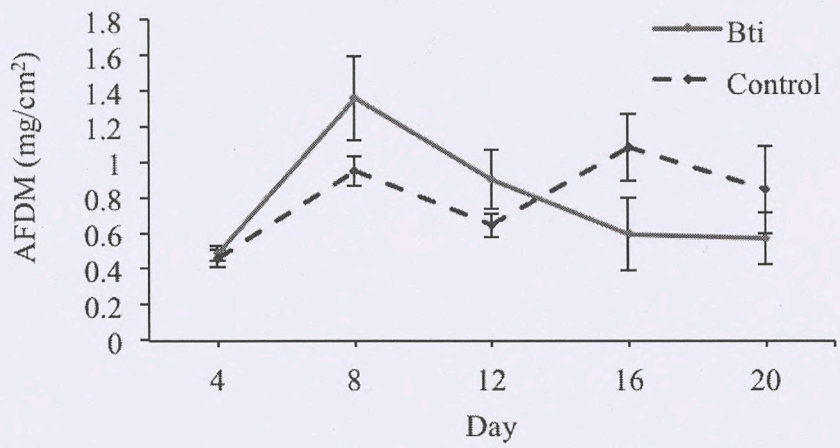

Figure 5. Mean ash-free dry mass concentrations in treatments and controls in trial one

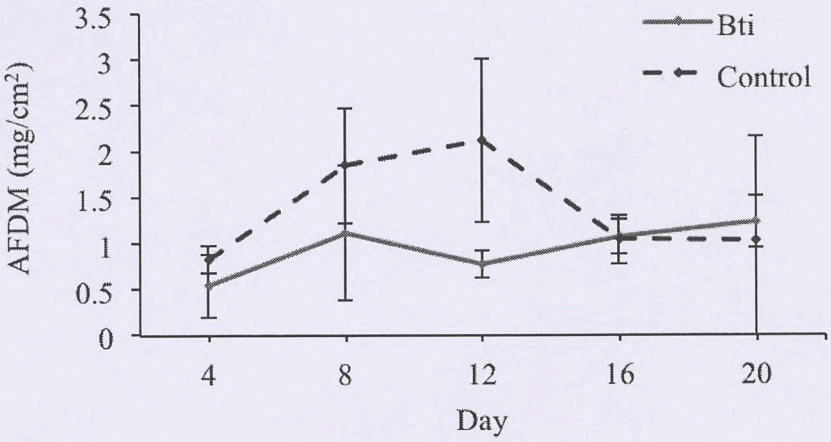

Figure 6. Mean ash-free dry mass concentrations in treatments and controls in trial two

APPENDIX F

Periphyton Biomass in Elevated and Ambient Temperature in ATS

Table 1. Water Temperature and Light Intensity in Treatments (Heated) and Controls (Non-heated)

Date	Day	Treatment	Flume	Temperature (°C)	Light (lum/m^2)
1/19/2016	4	Heated	1	22.9	2117.3
1/19/2016	4	Heated	3	23.0	3285.1
1/19/2016	4	Heated	5	22.7	3440.1
1/19/2016	4	Heated	8	22.7	3554.2
1/19/2016	4	Heated	9	22.8	3529.5
1/19/2016	4	Heated	12	23.0	3440.1
1/19/2016	4	Heated	13	23.0	3358.3
1/19/2016	4	Heated	16	22.9	2321.8
1/19/2016	4	Non-Heated	2	21.0	2907.3
1/19/2016	4	Non-Heated	4	21.0	3383.1
1/19/2016	4	Non-Heated	6	21.1	3487.5
1/19/2016	4	Non-Heated	7	21.1	3509.0
1/19/2016	4	Non-Heated	10	21.0	3549.9
1/19/2016	4	Non-Heated	11	20.9	3498.3
1/19/2016	4	Non-Heated	14	21.2	3206.6
1/19/2016	4	Non-Heated	15	21.1	2987.0
1/23/2016	8	Heated	1	22.7	1738.4
1/23/2016	8	Heated	3	22.9	3242.1
1/23/2016	8	Heated	5	22.7	3565.0
1/23/2016	8	Heated	8	22.9	3699.6
1/23/2016	8	Heated	9	22.7	3697.4
1/23/2016	8	Heated	12	22.9	3692.0
1/23/2016	8	Heated	13	22.8	3626.4
1/23/2016	8	Heated	16	22.8	2847.1
1/23/2016	8	Non-Heated	2	21.0	2729.7
1/23/2016	8	Non-Heated	4	21.1	3417.5
1/23/2016	8	Non-Heated	6	21.0	3650.0
1/23/2016	8	Non-Heated	7	21.0	3675.9
1/23/2016	8	Non-Heated	10	20.9	3736.1
1/23/2016	8	Non-Heated	11	21.1	3728.6
1/23/2016	8	Non-Heated	14	21.2	3542.4
1/23/2016	8	Non-Heated	15	20.9	3348.6
1/27/2016	12	Heated	1	22.9	1855.7
1/27/2016	12	Heated	3	23.0	3260.4
1/27/2016	12	Heated	5	22.9	3519.8
1/27/2016	12	Heated	8	22.8	3565.0

Date	Day	Treatment	Flume	Temperature (°C)	Light (lum/m^2)
1/27/2016	12	Heated	9	22.7	3568.2
1/27/2016	12	Heated	12	22.7	3571.5
1/27/2016	12	Heated	13	23.0	3525.2
1/27/2016	12	Heated	16	22.8	2601.6
1/27/2016	12	Non-Heated	2	21.0	2947.2
1/27/2016	12	Non-Heated	4	21.4	3404.6
1/27/2016	12	Non-Heated	6	21.2	3559.6
1/27/2016	12	Non-Heated	7	21.2	3535.9
1/27/2016	12	Non-Heated	10	21.0	3612.4
1/27/2016	12	Non-Heated	11	21.0	3602.7
1/27/2016	12	Non-Heated	14	20.9	3449.8
1/27/2016	12	Non-Heated	15	20.9	3228.1
1/31/2016	16	Heated	1	22.9	2123.7
1/31/2016	16	Heated	3	23.0	3357.3
1/31/2016	16	Heated	5	22.9	3618.8
1/31/2016	16	Heated	8	23.1	3637.1
1/31/2016	16	Heated	9	23.1	3651.1
1/31/2016	16	Heated	12	23.1	3569.3
1/31/2016	16	Heated	13	22.9	3484.3
1/31/2016	16	Heated	16	23.0	2561.8
1/31/2016	16	Non-Heated	2	21.3	3095.7
1/31/2016	16	Non-Heated	4	21.2	3565.0
1/31/2016	16	Non-Heated	6	21.3	3661.9
1/31/2016	16	Non-Heated	7	21.1	3696.3
1/31/2016	16	Non-Heated	10	21.1	3628.5
1/31/2016	16	Non-Heated	11	21.0	3609.1
1/31/2016	16	Non-Heated	14	21.0	3435.8
1/31/2016	16	Non-Heated	15	21.0	3178.6
2/4/2016	20	Heated	1	23.0	2148.5
2/4/2016	20	Heated	3	23.1	3328.2
2/4/2016	20	Heated	5	22.9	3551.0
2/4/2016	20	Heated	8	22.9	3632.8
2/4/2016	20	Heated	9	22.9	3621.0
2/4/2016	20	Heated	12	23.1	3579.0
2/4/2016	20	Heated	13	22.9	3517.6
2/4/2016	20	Heated	16	22.9	2309.9
2/4/2016	20	Non-Heated	2	21.4	2971.9
2/4/2016	20	Non-Heated	4	21.3	3439.1
2/4/2016	20	Non-Heated	6	21.1	3602.7
2/4/2016	20	Non-Heated	7	21.2	3610.2
2/4/2016	20	Non-Heated	10	20.9	3646.8
2/4/2016	20	Non-Heated	11	20.9	3625.3
2/4/2016	20	Non-Heated	14	21.0	3425.1
2/4/2016	20	Non-Heated	15	20.9	3166.7

Table 2. Corrected Chlorophyll a in Treatments (Heated) and Controls (Non-heated)

Date	Day	Treatment	Flume	Chl-a ($\mu g/cm^2$)
1/19/2016	4	Heated	1	0.4
1/19/2016	4	Heated	3	0.4
1/19/2016	4	Heated	5	0.6
1/19/2016	4	Heated	8	0.3
1/19/2016	4	Heated	9	0.6
1/19/2016	4	Heated	12	0.5
1/19/2016	4	Heated	13	0.2
1/19/2016	4	Heated	16	0.6
1/19/2016	4	Non-Heated	2	0.1
1/19/2016	4	Non-Heated	4	0.3
1/19/2016	4	Non-Heated	6	0.2
1/19/2016	4	Non-Heated	7	0.1
1/19/2016	4	Non-Heated	10	0.1
1/19/2016	4	Non-Heated	11	0.3
1/19/2016	4	Non-Heated	14	0.2
1/19/2016	4	Non-Heated	15	0.1
1/23/2016	8	Heated	1	13.8
1/23/2016	8	Heated	3	15.5
1/23/2016	8	Heated	5	5.8
1/23/2016	8	Heated	8	8.7
1/23/2016	8	Heated	9	11.1
1/23/2016	8	Heated	12	10.2
1/23/2016	8	Heated	13	6.7
1/23/2016	8	Heated	16	0.6
1/23/2016	8	Non-Heated	2	9.5
1/23/2016	8	Non-Heated	4	8.9
1/23/2016	8	Non-Heated	6	10.9
1/23/2016	8	Non-Heated	7	2.3
1/23/2016	8	Non-Heated	10	2.4
1/23/2016	8	Non-Heated	11	5.0
1/23/2016	8	Non-Heated	14	6.5
1/23/2016	8	Non-Heated	15	1.3
1/27/2016	12	Heated	1	13.1
1/27/2016	12	Heated	3	7.7
1/27/2016	12	Heated	5	18.0
1/27/2016	12	Heated	8	14.0
1/27/2016	12	Heated	9	6.8
1/27/2016	12	Heated	12	9.3
1/27/2016	12	Heated	13	4.8
1/27/2016	12	Heated	16	17.3
1/27/2016	12	Non-Heated	2	7.6
1/27/2016	12	Non-Heated	4	9.5
1/27/2016	12	Non-Heated	6	11.6

Date	Day	Treatment	Flume	Chl-a ($\mu g/cm^2$)
1/27/2016	12	Non-Heated	7	17.2
1/27/2016	12	Non-Heated	10	8.4
1/27/2016	12	Non-Heated	11	16.2
1/27/2016	12	Non-Heated	14	7.1
1/27/2016	12	Non-Heated	15	9.2
1/31/2016	16	Heated	1	5.0
1/31/2016	16	Heated	3	6.9
1/31/2016	16	Heated	5	10.1
1/31/2016	16	Heated	8	7.7
1/31/2016	16	Heated	9	15.2
1/31/2016	16	Heated	12	7.2
1/31/2016	16	Heated	13	14.5
1/31/2016	16	Heated	16	4.4
1/31/2016	16	Non-Heated	2	15.5
1/31/2016	16	Non-Heated	4	11.4
1/31/2016	16	Non-Heated	6	7.0
1/31/2016	16	Non-Heated	7	7.9
1/31/2016	16	Non-Heated	10	8.0
1/31/2016	16	Non-Heated	11	18.6
1/31/2016	16	Non-Heated	14	15.9
1/31/2016	16	Non-Heated	15	10.4
2/4/2016	20	Heated	1	16.3
2/4/2016	20	Heated	3	5.5
2/4/2016	20	Heated	5	6.9
2/4/2016	20	Heated	8	5.5
2/4/2016	20	Heated	9	6.1
2/4/2016	20	Heated	12	15.0
2/4/2016	20	Heated	13	7.9
2/4/2016	20	Heated	16	7.3
2/4/2016	20	Non-Heated	2	17.2
2/4/2016	20	Non-Heated	4	16.8
2/4/2016	20	Non-Heated	6	3.7
2/4/2016	20	Non-Heated	7	5.5
2/4/2016	20	Non-Heated	10	11.2
2/4/2016	20	Non-Heated	11	3.7
2/4/2016	20	Non-Heated	14	7.6
2/4/2016	20	Non-Heated	15	9.6

Table 3. Dry Mass and Ash Free Dry Mass in Treatments (Heated) and Controls (Non-Heated)

Date	Day	Treatment	Flume	DM (mg/cm^2)	AFDM (mg/cm^2)
1/19/2016	4	Heated	1	0.0378	0.0189
1/19/2016	4	Heated	3	0.0378	0.0378
1/19/2016	4	Heated	5	0.1134	0.0756
1/19/2016	4	Heated	8	0.0567	0.0378
1/19/2016	4	Heated	9	0.0189	0.0189
1/19/2016	4	Heated	12	0.0567	0.0189
1/19/2016	4	Heated	13	0.0378	0.0378
1/19/2016	4	Heated	16	0.0756	0.0378
1/19/2016	4	Non-Heated	2	0.0567	0.0378
1/19/2016	4	Non-Heated	4	0.0945	0.0756
1/19/2016	4	Non-Heated	6	0.0567	0.0378
1/19/2016	4	Non-Heated	7	0.0189	0.0189
1/19/2016	4	Non-Heated	10	0.0567	0.0378
1/19/2016	4	Non-Heated	11	0.0189	0.0189
1/19/2016	4	Non-Heated	14	0.0189	0.0189
1/19/2016	4	Non-Heated	15	0.0378	0.0189
1/23/2016	8	Heated	1	1.4178	0.8507
1/23/2016	8	Heated	3	1.1531	0.6805
1/23/2016	8	Heated	5	0.3214	0.2836
1/23/2016	8	Heated	8	0.4726	0.3781
1/23/2016	8	Heated	9	0.7183	0.4726
1/23/2016	8	Heated	12	1.0019	0.8318
1/23/2016	8	Heated	13	1.1153	0.9830
1/23/2016	8	Heated	16	0.1512	0.0945
1/23/2016	8	Non-Heated	2	0.8507	0.5671
1/23/2016	8	Non-Heated	4	0.6805	0.4348
1/23/2016	8	Non-Heated	6	0.9830	0.5860
1/23/2016	8	Non-Heated	7	0.4726	0.3214
1/23/2016	8	Non-Heated	10	0.4915	0.2079
1/23/2016	8	Non-Heated	11	1.4367	0.5671
1/23/2016	8	Non-Heated	14	0.9641	0.4726
1/23/2016	8	Non-Heated	15	0.2836	0.1323
1/27/2016	12	Heated	1	4.0832	3.2136
1/27/2016	12	Heated	3	1.1531	0.9452
1/27/2016	12	Heated	5	2.3440	1.8904
1/27/2016	12	Heated	8	1.2665	1.0964
1/27/2016	12	Heated	9	2.0605	1.8147
1/27/2016	12	Heated	12	1.2287	1.0019
1/27/2016	12	Heated	13	3.9319	2.6465
1/27/2016	12	Heated	16	0.5482	0.4159
1/27/2016	12	Non-Heated	2	2.7599	2.1739
1/27/2016	12	Non-Heated	4	1.9849	1.6446

Date	Day	Treatment	Flume	DM (mg/cm^2)	AFDM (mg/cm^2)
1/27/2016	12	Non-Heated	6	0.9641	0.8129
1/27/2016	12	Non-Heated	7	0.7372	0.6427
1/27/2016	12	Non-Heated	10	1.0586	0.9074
1/27/2016	12	Non-Heated	11	3.7996	3.0246
1/27/2016	12	Non-Heated	14	0.7372	0.6049
1/27/2016	12	Non-Heated	15	1.2287	0.8507
1/31/2016	16	Heated	1	2.6843	2.2306
1/31/2016	16	Heated	3	9.0548	7.7316
1/31/2016	16	Heated	5	1.8715	1.1342
1/31/2016	16	Heated	8	2.7788	2.4197
1/31/2016	16	Heated	9	1.4745	1.2854
1/31/2016	16	Heated	12	6.5028	6.4461
1/31/2016	16	Heated	13	1.4556	1.2287
1/31/2016	16	Heated	16	2.7788	2.5520
1/31/2016	16	Non-Heated	2	1.0019	0.6616
1/31/2016	16	Non-Heated	4	5.9546	4.7070
1/31/2016	16	Non-Heated	6	0.9263	0.6427
1/31/2016	16	Non-Heated	7	1.4745	1.1909
1/31/2016	16	Non-Heated	10	1.3422	1.1531
1/31/2016	16	Non-Heated	11	3.0246	2.3251
1/31/2016	16	Non-Heated	14	1.5312	1.2098
1/31/2016	16	Non-Heated	15	0.0189	0.1890
2/4/2016	20	Heated	1	2.6654	2.2117
2/4/2016	20	Heated	3	7.1456	5.8034
2/4/2016	20	Heated	5	2.5142	1.9849
2/4/2016	20	Heated	8	2.0794	1.6824
2/4/2016	20	Heated	9	5.2741	4.5369
2/4/2016	20	Heated	12	6.7486	6.2760
2/4/2016	20	Heated	13	2.4386	1.9471
2/4/2016	20	Heated	16	4.3478	4.0832
2/4/2016	20	Non-Heated	2	3.4026	2.8544
2/4/2016	20	Non-Heated	4	1.6635	1.4178
2/4/2016	20	Non-Heated	6	1.4745	1.2098
2/4/2016	20	Non-Heated	7	1.6068	1.4556
2/4/2016	20	Non-Heated	10	3.4405	3.1569
2/4/2016	20	Non-Heated	11	5.6900	5.1985
2/4/2016	20	Non-Heated	14	4.6692	4.1966
2/4/2016	20	Non-Heated	15	1.3043	1.1909